14 November 2024
EMA/101957/2025
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal assessment report

Cinainu

Common name: liquid ethanolic extract 30 per cent (W/W) of *Allium cepa* fresh bulb and *Citrus limon* fresh fruit / dry aqueous extract of *Paullinia cupana* seed / dry hydroethanolic extract of *Theobroma cacao* seed

Procedure No. EMEA/H/C/004155/0000

Note

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

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List of abbreviations

A/T	anagen/telogen
AA	Alopecia areata
ABC	Antibody binding capacity
AGA	Androgenetic alopecia
ANCOVA	Analysis of covariance
AR	Adverse reaction
ATC	Anatomical therapeutic chemical
ASMF	Active Substance Master File
AUC	Area under the curve
Bcl-2	B-cell lymphoma 2
CD1A	Cluster differentiation 1a
CDLQI	Children's Dermatology Life Quality Index
CI	Confidence interval
CI	Confidence interval
C_{max}	Maximum concentration
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Clinical Research Organisation
CS	Completers set
CSP	Centre Spécialités Pharmaceutiques
DER	Drug extract ratio
DMP	Data management plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EDV	Early discontinuation visit
EMA	European Medicines Agency
EQ-5D EuroQol	Five dimensions questionnaire
EQ-5D-Y	EuroQol five dimensions youth questionnaire
FDA	Food and Drug Administration
FPHL	Female-pattern hair loss
FU	Follow-up
GACP	good agricultural and collection practice
GCP	Good Clinical Practice
GLP	Good laboratory practice
GMP	Good manufacturing practices
HDPE	High density polyethylene
HMP	Herbal medicinal product
HSP	Heat shock protein
HUVEC	Human umbilical vein endothelial
ICF	Informed consent form
ICH	International Conference on Harmonisation
ICMR	Indian Council of Medical Research
IEC	Independent Ethics Committee

IL-8	Interleukin-8
IMP	Investigational Medicinal Product
IND	Investigational new drug
ISF	Investigator site file
ITT	Intent to treat
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LD	Lethal Dose
LOCF	Last observation carried forward
LS	Least square
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model for repeated measures
MPHL	Male-pattern hair loss
MTD	Maximal tolerated dose
NAAF	National Alopecia Areata Foundation
NCTC	National Collection of Type Cultures
NOEL	No observed effect level
p.o.	*per os* (oral)
p53	Tumor protein p53
PAAPBI	Paediatric alopecia areata patient benefit index
PDCO	Paediatric Committee (EMA)
PIP	Paediatric investigation plan
PPS	Per protocol set
PRO	Patient reported outcome
PT	Preferred term
REML	Restricted maximum likelihood
s.c.	Subcutaneous
SAE	Serious adverse event
SALT	Severity of alopecia tool
SAP	Statistical analysis plan
SAS®	Statistical analysis system
SDV	Source document verification
SOC	System organ class
SS	Safety set
SUSAR	Suspected unexpected serious adverse reaction
t½	Half-life
TEAE	Treatment-emergent adverse event
TLC	Think layer choromatography
Tmax	Time of maximum concentration
TMF	Trial master file
TNF alpha	Tumor necrosis factor alpha
V	Visit
VAS	Visual analogue scale
WHO	World health organization

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Legacy Healthcare (France) S.A.S. submitted on 24 March 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Cinainu, through the centralised procedure under Article 3 (2)(b) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 September 2018, which was based on demonstration of significant therapeutic innovation. The applicant applied for the following indication:

Treatment of moderate to severe alopecia areata in children and adolescents (from 2 to 18 years of age).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

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

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0220/2021 on the agreement of a paediatric investigation plan (PIP). At the time of submission of the application, the PIP P/0220/2021 was completed. The PDCO issued an opinion on compliance for the PIP P/0220/2021.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

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

1.5. Applicant's request for consideration

1.5.1. New active substance status

The applicant requested the active substances liquid ethanolic extract of *Allium cepa* (onion) fresh bulb and *Citrus limon* (lemon) fresh fruit / dry aqueous extract of *Paullinia cupana* (guarana) seed / dry hydroethanolic

extract of *Theobroma cacao* (cocoa) seed contained in the above medicinal product to be considered as a new active substances, as the applicant claims that they are not a constituent of a medicinal product previously authorised within the European Union.

1.6. Scientific advice

A strategic-regulatory meeting was held with EMA on 29 June 2016, to discuss the proposed change of strategy to prioritise the paediatric development for a MAA and postpone the MAA in the adult population. During this meeting, EMA pointed out that a concept with one pivotal study would need justification based on the points to consider on application with one pivotal study (https://www.ema.europa.eu/documents/scientific-guideline/points-consider-application-1meta-analyses-2one-pivotal-study_en.pdf) and a scientific advice request was recommended. However, the applicant did not request Scientific Advice / Protocol Assistance from the CHMP.

1.7. Steps taken for the assessment of the product

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

Rapporteur: Kristina Dunder Co-Rapporteur: Janet Koenig

The application was received by the EMA on	24 March 2023
The procedure started on	15 June 2023
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	4 September 2023
The CHMP Co-Rapporteur's critique was circulated to all CHMP and PRAC members on	18 September 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	18 September 2023
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	12 October 2023
The applicant submitted the responses to the CHMP consolidated List of Questions on	17 April 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	03 June 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 June 2024
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	27 June 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues	16 September 2024

on	
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	03 October 2024
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	10 October 2024
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	15 October 2024
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the Outstanding Issues following Oral explanation on	30 October 2024 07 November 2024
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Cinainu on	14 November 2024
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	14 November 2024

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Alopecia areata (AA) is an immune-mediated, inflammatory hair disease causing nonscarring hair loss in both, paediatric and adult patients. AA affects patients from all ethnicities, but more women than men, with similar clinical manifestations. It is a fluctuating disease, and a spontaneous remission is common, but such improvements become less frequent when patients suffer from the condition for a longer period, with several episodes of the disease. When AA starts early in infancy, its prognosis is usually more severe and associated with a higher frequency of unpredictable relapses during adulthood. The early factors responsible for the development of the autoimmune attack against hair follicles in AA remain still unanswered.

2.1.2. Epidemiology

AA is the second most common cause of hair loss following androgenetic alopecia, affecting 2% of the global population. AA appears to affect both genders equally and can occur in all age groups and ethnic backgrounds. AA is more prevalent in individuals with other autoimmune diseases.

2.1.3. Aetiology and pathogenesis

Although the exact aetiology of AA is not fully elucidated, it is recognised that the hair follicle bulb immune privilege collapse plays a critical role in the pathophysiology of the disease. The immunoinflammatory attack of scalp hair follicles abort normal hair cycling leading to their premature entry in telogen phase and hair loss. The disease starts with the occurrence of a perifollicular inflammation and invasion of immunoinflammatory cells around the anagen hair bulbs causing hair follicle immune privilege collapse, tissue dystrophy, hair follicle cell death, and hair shaft shedding. A central role is played by NKG2D + T cells and natural killer (NK) cells and autoreactive CD8+ T-lymphocytes that recognise autoantigens upon exposure of MHC I, II expression on follicular cells. T-cell activation is accompanied by an elevated INF-γ secretion both recruiting and activating more inflammatory cells including macrophages, mast cells, and dendritic cells leading to accelerated cell death and apoptosis of hair follicular cells.

2.1.4. Clinical presentation, diagnosis

The early clinical manifestations of AA appear as random, patchy hair loss, that may result sometimes in total scalp hair loss (alopecia totalis) or even become generalised to all body hair (alopecia universalis). Aside from scalp involvement, for severe forms, nail changes are also common. The evaluation of an AA patient includes medical and family history, examination of the scalp, the face and the entire body, including the nails, complemented by dermatoscopy. When clinical findings do not allow a definite diagnosis, additional investigations, such as a scalp biopsy, a fungal culture, or serology for other autoimmune diseases or infectious diseases may be necessary. To facilitate and standardise evaluation of the extent and course of AA, the Severity of Alopecia Tool (SALT) score was developed in 2004, which is based on a 4 pictures-based scoring system, where each side of the head is split into 4 quadrants, and scored by the investigator

accordingly, and a SALT score of 100% consisting of full-baldness. For centrally approved medicinal products, it has been agreed that a SALT score of ≥50 is considered severe AA. A definition of a moderate AA has not been established for a centrally approved medicinal product in the EU. The applicant considers a SALT score of 25-50 as a moderate AA.

Analyses have showed that 7% to 17% of adult and paediatric AA patients had depressive or anxiety disorders that require actual psychiatric care and medication. Impact of AA on children has been extensively documented. Among all autoimmune conditions, children with AA are the ones suffering the most from teasing and bullying, and they also suffer from poor health related quality of life (HRQoL), and notably, so do their parents.

2.1.5. Management

There are no centrally approved medicinal product for AA in children and no centrally approved medicinal product for moderate AA. Olumiant with the active substance baricitinib (JAK inhibitor) is authorised in the EU for the treatment of severe AA in adult patients (oral use). In 2023, Litfulo (ritlecitinib) was authorised for the treatment of severe alopecia areata in adults and adolescents 12 years of age and older (oral use). For children under 12 years of age, topical corticosteroids are being used.

2.2. About the product

Cinainu (also referred to as LH-8) is a cutaneous solution containing preparations of four herbal substances. It contains the combination of the following herbal preparations:

- liquid ethanolic extract of *Allium cepa* L. (fresh bulb) and *Citrus limon* (L.) Burm. f. (fresh fruit)
- dry hydroethanolic extract of *Theobroma cacao* L. (seed)
- dry aqueous extract of *Paullinia cupana* Kunth (seed)

The proposed indication is:

- treatment of moderate to severe alopecia areata in children and adolescents (from 2 to less than 18 years of age).

The proposed posology is:

- children from 2 to 15 years of age: 7 sprays of cutaneous solution to be applied to the whole scalp, twice daily;
- adolescents from 15 to less than 18 years of age: 8 sprays of cutaneous solution to be applied to the whole scalp, twice daily.

The product is to be spread on the whole scalp by massaging delicately with fingertips for at least 30 seconds. 30 minutes after application, the patient may wash and/or dry the hair. The proposed duration of use is a minimum of 3 months and should not be discontinued before 6 months, or once a stable response has been achieved.

Of note, the code "LH-8 cutaneous solution" allocated by the applicant for the paediatric development replaces code "CG 210", which was used in the initially and is referred to in some sections of the report.

2.3. Type of application and aspects on development

The CHMP did not agree to the applicant's request for an accelerated assessment. This was based on the limited number of study participants and non-robustness of the results presented from the RAAINBOW study in the request for accelerated assessment. The presented data planned for submission were considered insufficient to draw conclusions on the safety and efficacy of the product in the proposed indication for an accelerated assessment. In particular, the applicant's justification on the strength of evidence to support accelerated assessment was considered not acceptable. Thus, it was considered unlikely that the available data would lead to an earlier authorisation. Without sufficient safety and efficacy data at the time of submission and for which there are concerns about the strength of evidence, the request for accelerated assessment was rejected.

The proposed indication for Cinainu was:

"Treatment of moderate to severe alopecia areata in children and adolescents (from 2 to 18 years of age)"

Cinainu was proposed as a medicinal product subject to medical prescription and was supposed to be applied to the whole scalp of the patient, twice daily.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as a cutaneous solution containing three different herbals extracts as active substances: liquid ethanolic extract of *Allium cepa* (onion) fresh bulb and *Citrus limon* (lemon) fresh fruit / dry aqueous extract of *Paullinia cupana* (guarana) seed / dry hydroethanolic extract of *Theobroma cacao* (cocoa) seed.

The finished product contains ethanol 96 per cent and other ingredients.

The product was planned to be available in HDPE bottles, closed by a snapped measuring spray pusher pump delivering 0.13 mL per pressure, with a plastic over-cap, as described in section 6.5 of the SmPC.

The pump can be regarded as integral part of the medicinal product and the applicator a medical device (not an integral part but co-packaged). The device aspects are discussed further in the report.

Active substance liquid ethanolic extract of *Allium cepa* (onion) fresh bulb and *Citrus limon* (lemon) fresh fruit

General information

The active substance liquid ethanolic extract of *Allium cepa* (onion) fresh bulb and *Citrus limon* (lemon) fresh fruit is a herbal preparation manufactured using the following herbal substances: fresh bulb of onion, *Allium cepa* L., (with superficial scales removed) and peeled fresh fruit of lemon, *Citrus limon* (L.) Burm.f. The herbal extract is defined as a liquid ethanolic extract and the extraction solvent is ethanol 96% (v/v).

During the assessment procedure, several major objections were raised on the applicant's part of the Active Substance Master File (ASMF) supporting this active substance. The first major objection was raised on the

proposed name for the herbal preparation, asking the applicant to update the dossier with a herbal preparation name that reflects the extraction solvent as used during the manufacturing process. A second major objection was raised on the designation of the drug extract ratio (DER). The applicant was asked to amend the initially proposed two DERs and to bring the DER in line with the Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/CHMP/CVMP/287539 /2005 Rev.1). A third major objection was raised on the applicant's designation of the type of extract per Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/CHMP/CVMP/287539 /2005 Rev.1). Furthermore, a fourth major objection was raised on the need to declare the added excipient per relative weight ratio and its subsequent content in the composition of the extract. The applicant has addressed most of the issues regarding the objections to proposed name, DER and extraction solvent. The DER range should however be based on the results of the clinical batches to be suitable for the proposed product, but as the proposed range was further restricted within the clinical batch DER range, this is considered as acceptable. However, there are now two genuine DER's stated. As these do not exactly equate to one another, the applicant was asked to change all references of the genuine DER to one of ratio-to-1 (rather than %) as per regulatory standard. The major objection was resolved; however, this minor issue (other concern) was left open at the time of opinion.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF.

The active substance is obtained from a single source. Satisfactory GMP documentation has been provided for the active substance manufacturer.

The manufacturing method is a standard method for an ethanolic extract.

A major objection was raised during assessment as a number of documents from the current supplier were missing and a reference was made to a previous supplier. The applicant resolved this major objection by providing the correct documentation.

A number of major objections were raised on the manufacturing section of the restricted part of the ASMF. The difference in manufacture between clinical batch and validated batches of the proposed manufacturing process was not clearly presented. A detailed comparison of the manufacturing process, including in-process control data, as well as settings and durations of production steps was missing. Justifications for why each difference does not affect the quality of the herbal preparation was also missing. The applicant argued that the extract is not chemically diverse, because of the plant parts used. A metabolomic characterisation of the batches from the recent/intended manufacturing process was shown in support for this argument, and highlight the limited availability of analytical markers, identifying compounds and extensive fingerprint. However, without access to similar data on clinical batches at release, and with satisfactory stability for those, the quality of clinical batches and similarity with production batches are not ascertainable. The intended commercial and clinical batches have not been shown to be sufficiently similar in terms of characterisation, as critical specification items are not sufficiently comparable at relevant timepoints (release). Manufacturing methods are not sufficiently similar to be considered as basis for standardisation, as there are differences in the manufacturing processes of the extract that might impact the quality of the extract.

Furthermore, a major objection was raised on the fact that identification and assay have not been performed on the clinical batches. Therefore, comparability between clinical and intended commercial batches can not be assessed. To respond to this major objection, the applicant developed a bioassay in order to support comparability. However, at the time of conducting the comparability of the clinically used extracts and the new extracts, the clinical batches were expired. A new test of such expired batches at the present time was not accepted. The assay method for retroactive T0 on frozen samples has not been validated. The provided fingerprint method is incomplete without the inclusion of flavonoids, without which the current tests and acceptance limits for the stability study cannot neither differentiate a batch as expired or not, nor in terms of comparability over time. Regarding comparability of specification results, the analytical methods used at time of testing in the clinical batches were not validated and therefore not suitable for use in comparability exercise. A comparability of the batches with regards to regulatory requirements is no longer possible at the present time, as the clinical batches are expired. Therefore, the comparability on analytical data cannot be carried out. Claiming comparability based on results from the unvalidated bioassay, with relevance for bioactivity that has not been satisfactory substantiated, cannot be accepted as justification for stability of clinical batches. The bioassay results alone are not sufficient to support similarity of clinical and commercial batches.

Additionally, a major objection was raised on justification of differences in the manufacturing process of the clinical and commercial batches. Indeed, for herbal active substances, the consistent quality is assured by a narrowly defined manufacturing process to define a consistent quality of the active substance within the natural range of variation of the natural starting material. This requirement is not met for Cinainu.

These three major objections were unresolved at the time of opinion and are considered as part of a ground for refusal.

Moreover, major objections have been raised on missing dossier documentation, missing good agricultural and collection practice (GACP) confirmation for one of the lemon fruit suppliers, information on transport (including conditions) of lemon herbal substance, and details of the active substance manufacturing process. Another major objection was raised on the comparability of specifications of clinical and commercial batches with regards to a certain parameter. The applicant provided satisfactory responses to resolve these raised MOs.

Adequate in-process controls are applied during the active substance manufacture. The specifications and control methods for herbal substances, raw materials and reagents have been presented.

The active substance is packaged, and the primary packaging material complies with Commission Regulation (EU) 10/2011, as amended.

Specification

The active substance specification includes relevant tests.

Several major objections were raised on the specifications of the active substance.

The identification and assay of a marker s performed by HPLC using UV/Visible detection. The method is said to rely on HPLC retention time only. This was not considered satisfactory as identification solely by chromatographic retention time is not regarded as being specific. To resolve the major objection, the applicant complemented the identification by retention time by amending the method. This was found acceptable.

Two major objections were raised on the proposed reduced microbiological quality testing. In response the applicant updated the description of the manufacturing process by including information on herbal substances shipment and storage and tests information and provided acceptable certificates of analysis as well as justified the absence of microbial control on herbal substance, which was sufficient to resolve the major objections.

A further major objection was raised on a justification of acceptance criteria for assay, which is considered insufficient as the acceptance criteria should be derived from the clinical batches to ensure comparable and consistent quality of commercial batches. Furthermore, specificity of the identification methods needs to be proven, i.e. it must be ensured that the defined peaks are characteristic for the herbal drug. As the extract is obtained from two herbal substances, an additional fingerprint needs to be established covering a further characteristic chromatographic pattern of phytochemical constituents, which represents the multicomponent system next to specific markers, which are tested also in the assay. This is requested to ensure consistent quality for the multicomponent system in accordance with herbal Guidelines and in view of characterisation of the new active substance. In response, the applicant concludes that the markers can only be used as analytical markers for release specifications due to instability. Total markers between clinical and commercial batches are similar on release data. However, the clinical batches used a similar but unvalidated method, which is why justifications in comparability is said to take into consideration an additional 5% margin of error, which is statistically unsubstantiated. The applicant has developed identification and assay of an analytical marker for *Citrus limon* and the presence of a compound (ID for allium cepa) along with a fingerprint. These parameters have been tested on the first three batches retroactively on aged samples, all data conforms to specifications. The acceptance criteria for the parameter assay are still not justified. The acceptance criteria need to be derived from the clinical used batches as basis for the application. As another not validated analytical method was used for testing of markers in the clinical batches, the comparability assessment of results is not possible. The further statement, that the extract is to be defined as “other extracts” and therefore only analytical markers need to be selected, cannot be accepted as justification. The markers constitute a significant part of the extract composition and should be included in the fingerprint analysis, especially so since they have been considered in previous discussions on the justification of efficacy. Consequentially, a qualitative content of markers should be ensured at release and until the end of the storage period and in line with the extract used in the clinical batches. Although the fluid extract is re-classified as “other extract” the content of markers needs to be specified in accordance with the clinical batches. The consistent quality of the whole extract is the requirement for active substances used for medical products. Consistent quality should be ensured during stability of the extract and for the extract as used in the medicinal product. The major objection was not resolved, and it was made part of the grounds for refusal.

A major objection was raised on missing certificates of analysis covering all parameters of the specification. The applicant provided satisfactory certificates during the procedure thereby addressing and resolving the major objection.

An additional major objection was raised on the reference standards which were needed to enable quality control of the active substance. The applicant resolved this major objection by providing the requested data.

Batch analysis data on three commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Data is available for 3 commercial batches on long term and accelerated stability conditions. Based on the results the applicant concludes that their study supports an extrapolation for a proposed shelf-life. Regarding the presented stability data, two major objections were raised. First, neither of the two analytical markers used are meeting their specification acceptance criterium in the stability study. Analytical markers are meant to represent the stability of the herbal preparation. Based on this, the stability data does not support the proposed shelf-life. Furthermore, concerns are raised on the markers whose content diminishes for the herbal preparation already at the first post-release timepoint. Second, new ICH compliant data of at least 3 batches need to be submitted considering the above-mentioned. Considering that analytical methods and specification acceptance criteria have not yet been found satisfactory, the proposed shelf life is not accepted. This point is also put forward as a ground for refusal.

Active substance dry aqueous extract of *Paullinia cupana* (guarana) seed

General information

The active substance dry aqueous extract of *Paullinia cupana* (guarana) seed is a herbal preparation manufactured using the seed of *Paullinia cupana* Kunth (guarana) as herbal substance. The herbal extract is defined as a dry aqueous extract and classified as "other extract" based on the Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/CHMP/CVMP/287539 /2005 Rev.1), with an analytical marker and an excipient added as a carrier substance.

During the assessment procedure, several major objections were raised on the applicant's part of the Active Substance Master File (ASMF) supporting this active substance. The first major objection was raised on the initially proposed classification of the extract as a standardised herbal extract. Furthermore, the drug extract ratio (DER) must be defined and justified. The composition of the extract in respect to the native extract and excipient was requested. Furthermore, comparability between clinical and commercial batches was requested. This point was raised in the restricted part of the ASMF as well. In response, the applicant reclassified the extract as an "other extract", in line with the Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/CHMP/CVMP/287539 /2005 Rev.1) and in this respect the drug extract ratio (DER) has been defined and justified. This was sufficient to resolve the respective part of the major objection. To compare the previous clinical used standardised extracts and the new extracts applied for and manufactured under GMP a comparative list of data on the extracts was provided. However, based on the assessment and comparison of the manufacturing process, there are differences in the manufacturing process, whose impact on the final extract has not been fully investigated. Especially, as a lot of information on the previous clinical used extracts is missing, a comparison on important key parameters is not possible. The applicant's argument, that the specifications remain the same, cannot be accepted. For herbal medicinal products (HMPs) containing other extracts as active substance, the multicomponent system of the other extracts is defined to be the active substance. For herbal extracts, as multicomponent systems, the specification does only test on specific defined characteristics, however, does not contain a 100% analysis of the multicomponent system. This contrasts with chemical defined substances. Therefore, all parameters which could affect the multicomponent system need to be defined very narrowly to guarantee a reproducible manufacture of an extract that is as uniform as possible. This is necessary to obtain a comparable and reproducible

multicomponent system as active substance. Therefore, the identical starting material, extraction solvent and specially manufacturing process with identical conditions need to be used. This is not guaranteed for the newly named extracts in comparison to the clinically used batches. The comparability of clinical and commercial batches has not been demonstrated and considering that the clinical batches expired, comparability cannot be demonstrated. This aspect is included in a ground for refusal.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF.

The active substance is obtained from a single source. Satisfactory GMP documentation has been provided for the active substance manufacturer.

The manufacturing method is a standard method for a dry aqueous extract.

A major objection was raised during assessment on missing confirmation from both suppliers of the herbal substance, that all cultivation, collection and handling of the herbal substance, is compliant with GACP is requested. The confirmation was provided and found acceptable.

A number of major objections were raised on the manufacturing section of the restricted part of the ASMF. Further information on the handling of the herbal substance was required such as: treatment before and during harvesting (e.g., use of herbicides or pesticides), after harvesting (e.g., fumigation), details on drying as well as storage and transport conditions.

Both extract manufacturers and the manufacturing process have changed since the time of the RAAINBOW clinical trial, and it has not been demonstrated that the resulting preparations are comparable extracts. There are differences in the drug extract ratio as well as the manufacturing process and their impact on quality, safety and efficacy has neither been demonstrated nor justified. Considering the age of the clinical batches (expired) meaningful analytical comparisons are no longer possible and considering differences in the former and current manufacturing processes it has been demonstrated that a different extract is now produced. This major objection was unresolved at the time of opinion, and it is considered as part of a ground for refusal.

Further major objections raised on the restricted part include the missing details on the standard of water used in the manufacture of the active substance, compliance of the excipient to the Ph. Eur. monograph, details on steps of the manufacturing process, and missing chromatograms for identification parameters. The applicant resolved the major objections by providing data on the current manufacturing process, however it is noted that in some instances information on the process used to manufacture clinical batches could not be provided.

Adequate in-process controls are applied during the active substance manufacture. The specifications and control methods for herbal substances, raw materials and reagents have been presented.

The active substance is packaged in primary packaging material complying with regulatory requirements.

Specification

The active substance specification includes appropriate tests.

Several major objections have been raised on the suitability of the analytical procedures to control the herbal preparation and on the control of the active substance in general. Since identification and assay are considered validated for the herbal substance (Ph. Eur. methods from a specific monograph), descriptions and validations are required. The general method for heavy metals must be verified for specific substances. Furthermore, regarding the identification methods, the applicant is asked to justify how they can be considered stability indicating fingerprint chromatograms when used to control the herbal preparation in stability studies. The method is intended (and developed) to identify the herbal substance, if used for a different purpose than in the Ph. Eur. suitability for this purpose must be justified and demonstrated. The applicant should clarify whether the same methods are used by the finished product manufacturer. If other methods are used by the finished product manufacturer, they must also be described and validated. The applicant's claim that validations are not necessary for Ph. Eur. methods is a misunderstanding. Methods from the European Pharmacopoeia are only considered justified (and validated) when used for their intended purpose. I.e., methods from the specific Ph. Eur. monograph GUARANA are considered justified when used to control the herbal substance GUARANA, but not the herbal preparation dry aqueous extract from guarana seed. Batch size of the batches should be provided. Reference to general Ph. Eur. methods for testing on pesticides, aflatoxins and heavy metals is not sufficient. For aflatoxins, pesticides and heavy metals it is only a general analytical methods description in the Ph. Eur., which needs to be validated for the specific use. Therefore, in the specification the link to the specific analytical method needs to be included. An additional fingerprint needs to be established covering a further characteristic chromatographic pattern of phytochemical constituents, which represents the multicomponent system next to the marker, which is tested also in the assay. This is requested to ensure consistent quality for the multicomponent system in accordance to herbal Guidelines and in view of characterisation of the active substance. For the parameter assay, the following needs to be considered: for standardised herbal preparations, the content of constituents with known therapeutic activity must be indicated with the lowest possible tolerance. The acceptance criterion needs to be adapted and justified considering also clinical used batches. In the shelf-life specification, a variation in content of the marker of only ±5% from the initial batch-specific value is acceptable. For stability studies of herbal preparations with constituents of known therapeutic activity, the results obtained for the content of these constituents must be compliant with the release acceptance criterion (EMA/HMPC/CHMP/CVMP/201116/20051 Rev. 3). The shelf-life specification needs to be adapted accordingly. New CoA for at least 3 batches each for herbal substance and herbal preparation need to be provided considering the before stated issues. For numerical limits respective numerical results should be stated, this also applies to pesticides, aflatoxins and microbial quality. For chromatographic analytical methods the respective chromatograms need to be provided. Manufacturer/supplier and origin (for herbal substance), manufacturing date and batch size also need to be stated. The applicant provided the missing information and justifications thereby resolving the major objections; however several other concerns have remained open at the time of opinion - validation on assay for active substance extract from *Paullinia* is not sufficient and analytical methods cannot be accepted to be valid. The validated range of the marker assay is not considered as sufficient for the proposed acceptance limit; the validation of testing of heavy metals, mycotoxins are not acceptable as validation is not performed according to ICH Q2 and criteria set in 2.4.27. HEAVY METALS IN HERBAL DRUGS AND HERBAL DRUG PREPARATIONS. As particle size is a quality attribute of dry extracts, the parameter needs to be included in the specification. The data provided do not demonstrate consistent quality of the extract, as the particle size differs significant between the batches. It is further referred to guideline on herbal specification EMA/HMPC/CHMP/CVMP/162241/20051 Rev. 3. The applied range needs to be justified and should have been demonstrated to be suitable for the manufacturing process of the herbal medicinal product.

Batch analysis data on three commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Data is available for 3 commercial batches and based on the results the applicant concludes that all tests are compliant with the specifications and all time point, except one batch which is above the upper limit for assay at T9 (both long term and intermediate). Since the following time points are compliant this is acceptable. A re-test period is proposed. Regarding the presented stability data, four major objections were raised. First, there were concerns on the quality of method used for stability testing. In response, the applicant updated the method and resolved the major objection. Second, the results for the parameter assay show large variations within the shelf-life for the individual batches and are outside the specification for one batch at time point 9. Since no validation data are available for the analytical method so far, a final evaluation cannot be given in this regard. Third, the chromatograms are provided in different scales so that a comparability is not possible. Furthermore, the retention times of the main peak vary by several minutes which is to be ensured by respective robustness data in the validation report. Fourth, a second fingerprint is missing, which would give a statement on the stability of the multi-component system, especially as the extract is a new active substance. Therefore, a retest period cannot be derived from the data presented. Again, characterisation of new and expired clinical batches has been described and discussed in the report. Since the clinical batches were not analysed/characterised prior to the clinical trial, and the batches are now expired, it is still impossible to bridge the currently manufactured active substance batches with the clinical trial batches.

Active substance dry hydroethanolic extract of *Theobroma cacao* (cocoa) seed

General information

The active substance dry hydroethanolic extract of *Theobroma cacao* (cocoa) seed is an herbal preparation manufactured using the seed of *Theobroma cacao* L. (cocoa) as herbal substance.

The herbal extract is defined as a dry hydroethanolic extract. According to the Applicant, the extract is not adjusted to a particular content of constituents to obtain the correct levels of analytical markers. As such, the extract could be defined as an 'other extract' based on the Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/CHMP/CVMP/287539 /2005 Rev.1). However, the extract can neither be classified as an 'other extract' (as it is adjusted for marker content) or as a 'standardised extract', as it has not been demonstrated/justified that the marker is a "constituent with known therapeutic activity". An excipient is specified as a carrier however, the final amount to be added is not yet established, suggesting that the manufacturing process is not yet finalised. This applies also to the large difference in DER proposed for the commercial batches compared to what has been used for clinical batches, where the applicant claims that further optimisation of the manufacturing process is needed. Further changes include the removal of carrier that was used for clinical batches in addition to the excipient, addition of a step in the commercial batch manufacturing process, as well as two steps where heat is applied. No information on elevated temperatures is provided for the extract used for clinical batches, and it cannot be excluded that this might have had an impact on the extract as a whole, as compared to the extract used for clinical batches. Finally, the limits of

the marker substances in the extract have been modified in the commercial as compared to the clinical batches of the extract. A major objection has been raised on the applicant's part of the Active Substance Master File (ASMF) on inadequate comparability data to support the changes in the extract and its manufacture from clinical to commercial batches. Further major objections have been raised on the restricted part of the ASMF on these aspects. This point is put forward as a ground for refusal. With the complexity of herbal preparations being used as active substances, it is essential that the manufacturing process after a transfer is the same, as the extract is the active substance. The applicant has demonstrated that they do not possess detailed knowledge about several relevant manufacturing steps of the clinical batches. Consequentially, relevant knowledge for such a transfer is evidently missing and cannot be replaced by assumptions. Despite the data that the applicant has provided, it is evident (e.g. from the different TLC chromatograms of the non-GMP and the GMP extract; the large difference in DER) that the extracts are different from each other, and hence a bridge between the applied product and the clinical product cannot be made.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF.

The active substance is obtained from a single source. Satisfactory GMP documentation has been provided for the active substance manufacturer.

The manufacturing method is a standard method for a dry hydroethanolic extract. Dried *Theobroma cacao* L. seeds are extracted with hydroethanolic (30 per cent V/V) solvent. It is then packaged.

Concerns on the data to support the change in the manufacturing process used to manufacture clinical batches to the commercial one are discussed earlier in this report.

Adequate in-process controls are applied during the active substance manufacture. The specifications and control methods for herbal substances, raw materials and reagents have been presented.

The active substance is packaged in the primary packaging material complying with regulatory requirements.

Specification

The active substance specification includes appropriate tests

A major objection was raised on the need for the specifications of the herbal substance to comply with the Ph. Eur. monograph Herbal Drugs (1433). During the procedure the addition of compliance to Ph. Eur. monograph Herbal Drugs (1433) in the specification of the herbal substance is acknowledged. However, a test for assay (or the justification and authorisation of its absence) is still missing in the specification. Additionally, the provided validations for the analytical methods are partly very brief and several of them are not performed according to the guideline ICH Q2(R2) Validation of analytical procedures. This point was also put forward as a part of grounds for refusal.

Batch analysis data on one pilot batch and one commercial batch of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from one pilot batch and one commercial batch of active substance from the proposed manufacturer stored in the intended commercial package under long term, under intermediate conditions and under accelerated conditions according to the ICH guidelines were provided.

However, a major objection was raised asking for data on more batches to be generated prior to assigning a retest period. The major objection was unresolved at the time of opinion.

2.4.2. Finished medicinal product

2.4.2.1. Description of the product and pharmaceutical development

The finished product is a herbal medicinal product supplied as a cutaneous solution.

The goal of pharmaceutical development was to manufacture (under GMP) a stable cutaneous solution containing three herbal preparations (extracts) as active substances (liquid ethanolic extract of *Allium cepa* (onion) fresh bulb and *Citrus limon* (lemon) fresh fruit / dry aqueous extract of *Paullinia cupana* (guarana) seed / dry hydroethanolic extract of *Theobroma cacao* (cocoa) seed), appropriate for the intended use. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards, except an excipient for which acceptable specifications and analytical methods have been provided. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

Unlike novel medicinal products which generally stand on the handful of industrial batches, LH-8 cutaneous solution traces back to an extensive industrial history of over 10 years, 115 industrial batches and 2.6 million units sold.

In the dossier, the applicant discusses how content of different components from the included extracts has been established. However, this has been based mainly on historical data and not on clinical trial batches form the clinical trial. The component from one extract was not even monitored in the clinical trial batches, and another group were at the time of the clinical trial quantified with a non-validated method. In addition to this, extract manufacturers have changed since the clinical trial and in conjunction with this, details in the manufacturing processes. Commercial batches and clinical batches have not been demonstrated as comparable. Also, since it hasn't been justified that monitored components are "constituents of known therapeutic activity" (i.e., that the extracts are standardised), it is not sufficient to base comparability on quantifying those compounds only. The applicant has proposed within the procedure to re-define the contained active substance extracts as "other extracts". However, the previous and concurrent applied manufacturing process and control strategy do not correspond to a herbal medicinal product that contains "other extracts" as active substances. More elaborate quality control strategies (e.g., fingerprint chromatograms) would be necessary for the extracts now defined as "other extracts". A major objection was raised in this regard, and since it was unresolved at the time of opinion, it is considered one of the grounds for refusal: the applicant could not demonstrate that the medicinal product now applied for corresponded to what was tested in the single pivotal clinical study (the RAAINBOW study). In addition, the applicant could not demonstrate that the manufacturing process of clinical batches and current batches is comparable and can ensure a consistent quality. Differences in the manufacture of clinical batches and current batches have been identified but not adequately justified by the applicant. Furthermore, there are significant differences in the pivotal attribute drug extract ratio (DER) for the guarana and cacao extracts. In the responses trying to

resolve the identified major objection, the applicant refers to a bioassay and tries to demonstrate comparability between clinical batches and commercial batches, as well as stability for the product, through evaluation of the clinical efficacy. According to the applicant, efficacy has been demonstrated for 3 batches of different age. Based on the development of fingerprint batches, the applicant considers that the changes observed in stability studies are supposed to occur. However, the proposed applicant's concepts are not supported by the EU regulatory framework for herbal medicinal products. The clinical relevance of a bioassay detail has not been comprehensibly elaborated by the applicant. A consistent link between the clinical trial batches and the current herbal medicinal product batches or the age of these batches in regard to the bioassay activity and overall efficacy cannot be drawn. An attempt was made to develop a medicinal product retrospective after a clinical trial, based on assumptions about the quality of the herbal medicinal product in a clinical trial. Instead, investigation of the clinical efficacy (and safety) of a developed product with quality characterised by appropriate analytical methods should have been performed. In summary, comparability between batches used for the clinical trial and commercial batches has not been demonstrated. On the contrary, it has now been demonstrated that the clinical batches contained different extracts (big difference in drug extract ratio). Furthermore, analytical procedures used at the time of the trial were not validated and because of this the clinical trial batches that are now expired were not sufficiently characterised, which means that it is impossible to demonstrate comparability. Since the clinical batches are now expired, any further analyses are therefore meaningless. Additionally, the stability of the finished product cannot be demonstrated through "clinical efficacy". It should be the other way around, the purpose of stability studies is to, based on analytical tools, indicate when the product is expired and no longer have the intended effect.

The primary packaging is a white HDPE bottle, closed by a snapped-on measuring and protected with a white PP cap. The bottle is packed in a cardboard box containing a leaflet and a PP applicator (nozzle). The primary packaging material complies with Ph.Eur. and EC requirements. The pump can be regarded as integral part of the medicinal product and the applicator a medical device (not an integral part but co-packaged).

Based on MDCG 2022-5 Guidance on borderline between medical devices and medicinal products under Regulation 2017/745 on medical devices, the pump is an integral product intended exclusively for use in the given combination and is not reusable.

The co-packed applicator is (2017/745, Section 4.2, Chapter III, Annex VIII, Rule 2), a class I device.

Furthermore, according to EMA Questions & Answers on Medical Devices, https://www.ema.europa.eu/documents/regulatory-procedural-guideline/questions-answers-implementation-medical-devices-vitro-diagnostic-medical-devices-regulations-eu/745-eu-2017/746_en.pdf: "Some devices are not considered to form an integral product with the medicinal product and are treated as a container closure system e.g. nozzle on the top of the container for eye drops, syringe for reconstitution (without purpose for administration of the medicinal product) or can be treated as an excipient e.g. transdermal patches (using passive diffusion), in which case, they do not fall under the second subparagraphs of Article 1(8) or (9) MDR and hence, do not need to comply with Section 3.2., point 12, of Annex I of Directive 2001/83/EC, as amended by Article 117 MDR."

It is considered that this is applicable for the pump and applicator for the finished product and that no further information such as CE marking or opinion from a Notified Body is required.

2.4.2.2. Manufacture of the product and process controls

Satisfactory GMP documentation has been provided to demonstrate GMP compliance of the manufacturer.

The manufacturing process is a standard process for cutaneous solutions, consisting of four main steps: blending of the active substances (extracts) with excipients, pH adjustment (if necessary), filtration and packaging.

Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

2.4.2.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form.

During the assessment several major objections have been identified on the control strategy of the finished product. Since the product is a Herbal Medicinal Product, the applicant was asked to refer to the following guidelines: Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/162241/2005 Rev. 3), Guideline on quality of herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/201116/2005 Rev. 3), as well as the following reflection paper: Reflection paper on markers used for quantitative and qualitative analysis of Herbal Medicinal Products and Traditional Herbal Medicinal Products (EMEA/HMPC/253629/2007). First, the acceptance criteria for the assays cannot be accepted. Since the application is based on a clinical trial, the acceptance criteria must be set based on the content of the batches used in the trial (i.e. "clinical trial batches"). This is not the case and the CHMP cannot see how it would be possible, considering that some compounds were not monitored in the clinical trial batches and for, some, the analytical procedure was not validated and therefore, the results cannot be considered convincing.

There is also a major issue with the analysed compounds since it is questionable whether they are "constituents of known therapeutic activity" (i.e., a criterion for being defined as standardised extracts), or if they rather should be classified as active markers or analytical markers. For standardised extracts, it could be acceptable to assay only the constituents of known therapeutic activity (albeit fingerprint chromatograms are required for identification), but for quantified extracts (containing active markers) and "other extracts" (containing analytical markers), the extracts ("in their entirety") are considered the active ingredients. Therefore, it is not sufficient to quantify the marker substances from each extract, the extracts must be quantified as well (based on "batch specific" data from the extracts used in the specific batch of the product).

The proposed very wide release limits for the assays of marker substances (i.e., for quantified and "other" extracts) could be acceptable if the results (by batch specific calculations) are used to certify that the quantity of each extract (i.e., the "active substances") is constantly (during shelf life) and reproducibly (at release) held within much tighter limits that are normally acceptable for medicinal products. Usually, this should be ± 5 %, but can sometimes for herbal medicinal products (please refer to the above-mentioned guidelines), if acceptably justified, be widened to ± 10 %. Furthermore, it is important that the quantity of the extracts is within ± 10 % of the initial value during shelf life.

Markers should be suitable for their intended purpose (e.g. identification, quantification, analytical control, stability). Based on observations from stability studies, a marker is clearly not stable and the suitability as a marker is therefore questioned by the CHMP.

The acceptance criteria for a marker cannot be accepted without an upper limit. Also, the limits of the specification must be covered by the methods validated range which is not the case. The acceptance criteria must be updated.

Furthermore, the limits for marker are not covered by the methods validated range.

If the clinical trial batches have not been sufficiently characterised and the newly produced medicinal product batches can therefore not be sufficiently compared with the clinical trial batches, a fundamental problem exists, which prevents a successful marketing authorisation. In this specific case, the issue is further complicated by the observation that high variability and obvious instability of the herbal medicinal product is observed within a short time after release. In combination with the fact that the clinical trial batches where not sufficiently characterised during the clinical trials it appears to be rather impossible at this time point to identify the quality of the clinical trial batches, which did exist at the time point of the clinical trials.

In this regard, it is also highlighted that the provided metabolome/fingerprint provide only little informational value. The analysed clinical trial batches had an age of 57 months in the "Analysis of Coacillium[1] cutaneous solution composition" study and in the "Comparisons of UPLC-PDA profiles of clinical, stability and intended product batches" study by SNH the analysed clinical trial batches had an age of 57-97 months at the time point of the analyses. Such studies would have more informational value if they would have been conducted during the clinical trials.

The analyses long time after the clinical trials and after the proposed shelf life cannot be used to characterise the clinical trial batches in order to identify the quality that should be achieved by the now produced herbal medicinal product. It is not reasonable if the newly produced herbal medicinal product batches are produced and optimised in order to be comparable with batches that have expired. The expired batches do probably no longer possess the same quality and postulated efficacy of the clinical trial batches during the clinical trials.

The applicant has provided a nitrosamine risk assessment. The overall conclusion of the applicant in the template is that there is a risk of presence of nitrosamines. However, the risk is not identified, and all references state "will be provided when available", and the template only indicates whether root causes are evaluated or not, outcomes of evaluation (i.e., whether there is a risk or not) is not included. The risk assessment is only preliminary, and no conclusion has been drawn. The final risk assessment should contain further assessment of the excipient, the acidic pH as well as an investigation of the root cause of formation of the detected nitrosamine. Monitoring of this nitrosamine has not been specified as control at release and stability testing. Also, monitoring of this nitrosamine should be included in future stability studies. Please note that post-approval commitments are normally not acceptable for nitrosamine risk assessments (in line with CMDh/412/2019). This point is also included in the grounds for refusal.

Another major objection was raised on the need to restrict the specification limits for impurities. Certain impurities need to be restricted and controlled in the HMP specification. Skip testing of pyrrolizidine alkaloids can only be applied if sufficient data is available to support skip testing, which is not the case. This major objection was also unresolved at the time of opinion.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on 3 batches was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is

[1] Previous name of the applied product Cinainu

not necessary to include any elemental impurity in the finished product specification. The information on the control of elemental impurities is satisfactory.

Batch analysis results are provided based on the initially proposed specifications. The certificates of analyses in line with the revised specifications are pending at the time of opinion.

2.4.2.4. Stability of the product

Since there are outstanding issues on the control of the finished product and finished product specifications, no shelf life can be established. According to the applicant, the clinical trial has been conducted with batches that have been 7-27 months old. The last treatment was conducted with 35-36 months old batches. At the end of the clinical trials the contained herbal preparations were up to 38-54 months old. In consideration of the observed instability of the pharmaceutical preparation, this comprises further issues. For a marketing authorisation, it needs to be possible that a reproducible and consistent quality of the pharmaceutical preparation and the contained herbal preparations can be specified and ensured from the beginning until the end of the proposed shelf life. The analytical assessment of the clinical trial batches is deficient. It can no longer be retraced if batches analysed at release are comparable with 36 months old batches as the clinical batches are expired. This hampers or makes it even impossible to deduce a relevant analytical profile from the clinical trial batches.

The proposed applicant's concept of a "natural evolution of the herbal medicinal product" is also not supported by the regulatory EU frame work for herbal medicinal products. Also, herbal medicinal products need to possess a reproducible quality at release and until the end of the proposed shelf life. In cases where the herbal substance/herbal preparation does not have constituents with known therapeutic activity simply determining the stability of active markers or analytical markers, will not suffice and a series of stability-indicating tests (e.g. TLC, HPLC) will be needed. The stability of the herbal substance/herbal preparation as a multi-component system, should, as far as possible, also be demonstrated, e.g. by means of appropriate fingerprint chromatograms.

It was also not demonstrated that their proportional content remains comparable to the initial chromatographic fingerprint. It is considered acceptable to start the stability studies with an herbal substance/herbal preparation/HMP up to three months after the manufacturing date. However, in case extensive instability occurs during the first three months, start of the stability studies 3 months after the manufacturing date is not acceptable.

In conclusion, it is not acceptable if significant degradation during the shelf life of the herbal medicinal product occurs. Especially for a herbal medicinal product that contains "other extracts" as active substances it is not acceptable to ignore such a degradation of putatively relevant component fractions. In this case, it is not known which compounds are important for the efficacy of the herbal medicinal product or which quantities need to be assured of each compound. In this case, the quality and composition need to be deduced by the quality and composition of the herbal medicinal during the clinical trials. It is referred to the current herbal quality guideline EMA/HMPC/CHMP/CVMP/201116/20051 Rev. 3. For the assessment about the provided bio-assay data it is noted that this assay is not part of the specification and is not further assessed here.

Overall, it can be concluded that, inter alia based on degradation of flavonoids, the product is not stable for more than a few weeks when stored below 25 °C. No shelf life can be established. This major objection is unresolved and made part of grounds for refusal.

2.4.2.5. Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.3. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has not been presented in a satisfactory manner. A large number of major objections have been identified and not satisfactorily addressed by the Applicant. Based on these, the following quality grounds for refusal have been formulated:

- The comparability between the finished product proposed for marketing and the clinical batches studied in the single pivotal clinical study (RAAINBOW trial) was not demonstrated. In addition, the applicant could not demonstrate that the manufacturing process of clinical batches and intended commercial batches is comparable and can ensure a consistent product quality. Differences in the manufacture of clinical batches and intended commercial batches have been identified but not adequately justified by the applicant. Furthermore, there are significant differences in the pivotal attribute drug extract ratio (DER) for the guarana and cacao extracts between the batches used in the single pivotal clinical study and the batches proposed for marketing.
- Stability is not demonstrated for the proposed finished product. The bioassay proposed by the applicant cannot be considered an adequate analytical tool to demonstrate stability and cannot be used to establish a shelf-life of the finished product. Supportive data might be used in the stability studies, but the bioassay proposed cannot replace stability studies under ICH conditions.
- Major deficiencies regarding the quality control of all extracts and the finished product (acceptance criteria for several specification parameters are not agreed, comprehensive validation of relevant analytical procedures was not provided, etc.) have been identified. The requirements of scientific guidelines regarding the development of medicinal products (i.e. Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/CHMP/CVMP/287539 /2005 Rev.1), Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/162241/2005 Rev. 3), Guideline on quality of herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/201116/2005 Rev. 3), as well as the following reflection paper: Reflection paper on markers used for quantitative and qualitative analysis of Herbal Medicinal Products and Traditional Herbal Medicinal Products (EMEA/HMPC/253629/2007)) have not been followed.
- The final results of the risk assessment of nitrosamines have not been submitted. The overall conclusion of the applicant is that there is a risk of presence of nitrosamines. However, the root cause for this risk is not identified. The final risk assessment provided by the applicant is missing further assessment of the excipient, the acidic pH as well as an investigation of the formation of the detected nitrosamine. Also, monitoring of this nitrosamine is not included in future stability studies. Monitoring of this nitrosamine has not been specified as control at release and stability testing.

2.4.4. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is not considered acceptable and quality grounds for refusal are formulated.

2.4.5. Recommendations for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Introduction

The non-clinical documentation was mainly based on a bibliographical review of published information on the well-known herbal active substances which are part of the proposed medicinal product, and the applicant also included bibliographic data on three markers of the extracts: quercetin, caffein and theobromine. Further proprietary data were generated by the applicant concerning:

- The toxicity of the proposed medicinal product by the oral route in rats.
- The *in vitro* genotoxicity of the herbal active substances.
- The *in vivo* genotoxicity of the proposed medicinal product.
- The local tolerance of the cutaneous solution.
- Repeat-dose toxicity of the proposed medicinal product by dermal administration to mini-pig for 28 days.

In general, herbal extracts contain a multitude of active compounds. The applicant performed an analysis of the complete content of the product and provided a list of identified compounds, however, it does not contain a quantification of the identified compounds. It was not identified which of the compounds are of pharmacological and/or toxicological interest and no information on the pharmacokinetics of the compounds present in relevant concentrations was presented. While waiving certain studies or replacing original studies by literature data could be acceptable as long as justified, the applicant did not compile a complete dossier regarding the dermal application of these extracts, and did not fully justify the lack of certain data.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

The claimed mechanisms of action for this proposed medicinal product in AA are at the hair follicle:

- Anti-inflammatory effects
- Inhibition of premature apoptosis

These properties were described in the submitted literature for the assumed active constituents of the finished product cutaneous solution and the applicant submitted *in vitro* studies performed with the finished product cutaneous solution.

Plant		Documented therapeutic effect
Allium cepa	Anti-inflammatory, antioxidant properties	
	Immunomodulatory effects of flavonoids	
	Antimicrobial activity	

	Prevention and treatment of alopecia areata with quercetin in the C3H/HeJ mouse model	
		Use of topical onion juice for alopecia areata
Citrus limon	Anti-inflammatory, antioxidant, antibacterial	
	Stabilization of flavonoids at acidic pH	
Theobroma cacao	Antioxidant and anti-inflammatory action by the inhibition of PDE4 by theobromine	
	Stimulation of flavonoid transport in cells	
		Immunomodulatory properties of cacao extracts, potential consequences for medical applications
Paullinia Cupana		Effect of caffeine and testosterone on the proliferation of human hair follicles in vitro
		Antioxidant and anti-inflammatory action by the inhibition of PDE4 by caffeine
		Phosphodiesterase 4-targeted treatments for autoimmune diseases
		Anti-inflammatory effect and inhibition of allergic reaction by Paullinia cupana seed extract
		Low concentrations of caffeine have anti-apoptotic activities, free radical scavenging capabilities, and antioxidant functions

Anti-inflammatory effects:

Studies conducted by the applicant

CG 210: An *in vitro* study was claimed to evidence the efficacy of the product (named Compound C/CG210 in the study report), demonstrating the potential of this product. The expression levels were analysed using specific Antibody Binding Capacity (sABC) technology.

LH-8 (D0T86) study MV210308: The effects of the clinical product (i.e. product batch used in the clinical study) was evaluated.

LH-8 study ST240204: The effects of the intended product, the clinical product and the stability batch, at different timepoints after production were evaluated. Under the experimental conditions of the assay, compound (batch type: stability) displayed observed properties.

Bibliographic data

Quercetin: Some *in vivo* studies demonstrating the anti-inflammatory activity of quercetin were identified, although none after cutaneous application.

In carrageenan-induced acute inflammation in adult male albino rats, oral administration of quercetin at 50 mg per kg for 14 consecutive days before induction of acute inflammation showed anti-inflammatory effects at all timepoints after carrageenan injection. In addition, pre-treatment with quercetin significantly prevented elevation of malondialdehyde and nitric oxide, improved the loss of GSH (reduced glutathione) ($p<0.05$) and reduced TNF-α release (Heeba, 2014). In arthritis-induced male Lewis rats, increases in selected markers of inflammation such as IL-1β ($p<0.001$), and MCP-1 ($p<0.01$) were ameliorated by oral administration of quercetin (150 mg/kg bodyweight) for 28 days compared with untreated arthritis-induced rats (Gardi, 2015).

A herbal fraction of A. cepa bulb containing 7 % quercetin given orally at 200 mg/kg, before injection of carrageenan showed a significant anti oedematogenic effect in the paw oedema test (Kaiser, 2009). A microemulsion of 0.3% w/w Quercetin decreased UV irradiation induced NF-κB DNA-binding by 80% in primary human keratinocytes. Consequently, quercetin suppressed UV irradiation-induced expression of inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α in vitro (Vicentini, 2010).

Theobroma cacao L. seed extracts and extracted substances: *In vitro* studies suggest the anti-inflammatory properties of various *T. cacao* extracts against inflammatory markers. In human PBMC, an aqueous extract of *T. cacao* L. (5 and 10 µg/mL) significantly inhibited phyto haemagglutinin (PHA)-induced INF-γ secretion ($p<0.05$) vs PHA-stimulated cells (Jenny, 2009). In macrophage cell lines, 50 µg/mL of an ethanolic extract of *T. cacao* (containing 20.4 mg/mL total polyphenols) significantly diminished MCP-1 secretion ($p<0.01$) and TNF-α secretion ($p<0.05$) induced by LPS (Ramiro, 2005). In addition, pre-treatment of macrophage with the extract, decreased TNF-α, IL-1α and IL-6 mRNA expression l. In HUVECs, treatment with concentrations of 25, 50 and 100 ppm of an ethanolic extract of *T. cacao* prevented the increase in IL-6 and sVCAM-1 induced by the addition of plasma from preeclamptic patients ($p<0.05$) (Rahayu, 2016). Phenolic extract from unroasted and roasted cocoa at the concentrations of 1 and 10 µg/mL, which significantly inhibited PMA-induced TNF-α release ($p<0.05$), and PMA-induced IL-6 release ($p<0.05$) (Zeng, 2011). In human monocytes, clovamide (a polyphenol extracted from *T. cacao* L. seeds) at 10 and 100 nM inhibited phorbol 12-myristate 13-acetate (PMA)-induced TNF-α release ($p<0.001$) as well as PMA-induced IL-6 release ($p<0.01$).

Inhibition of apoptosis (bibliographic data):

Caffeine: In an *in vitro* study, caffeine significantly reduced DNA crosslinking induced by 5-methoxypsoralen in association with UVC treatment, in human lymphocytes. This study showed that caffeine can reduce phototoxicity (Szeto, 2010).

2.5.2.2. Secondary pharmacodynamic studies

No data have been submitted on product specific studies. Some bibliographic data regarding the secondary pharmacology of the main active substances and their main biomarkers for activity (quercetin, caffeine and theobromine) have been provided. The main pharmacological properties, largely described after oral administration or in experimental *in vitro* studies, identified as not directly related to the therapeutic indication are, according to the applicant:

- Antioxidant activity, probably in relation to the presence of quercetin and caffeine.
- Effect on the CNS attributed to the presence of *Citrus limon*, caffeine and theobromine.
- Effects on the cardiovascular system, due to the presence of caffeine and theobromine.

In vitro, in human lymphocytes, pre-treatment with quercetin (10, 50 and 100 µM) leads to a significant decrease in H2O2-induced oxidative damage and a dose-dependent protective effect against formation of DNA adducts (Wilms, 2005). After oxidant treatment of 3T3-L1 cells with ferric ammonium citrate, a concentration of 2 µg/mL of an ethanolic dry extract of *Paullinia cupana* seed (var. sorbilis Mart) reduced lipid peroxidation by 65.2%, as measured by the malonyl dialdehyde test (Basile, 2005; ESCOP, 2009).

2.5.2.3. Safety pharmacology programme

No specific safety pharmacology study was performed with the cutaneous solution, or its components and no data was identified from the literature review by the applicant. Potential effects on the CNS and CVS were presented:

Effect on the CNS (bibliographic data):

Citrus limon juice and quercetin: In adult albino mice, oral administration of fresh and filtered juice of *Citrus limon* was effective in improving learning and memory (Riaz, 2014). In male Mongolian gerbils, oral administration of 50 mg/kg of an onion extract in 80 % ethanol containing 2.19 mg of quercetin / g of extract showed a neuroprotective effect (Hwang, 2009). In a review published in 2015, Kawabata et al. concluded that quercetin and its metabolites may prevent and/or improve mood disorders by exerting antioxidant and anti-stress activities in the brain (Kawabata, 2015).

Paullinia cupana Kunth and caffeine: In the forced swimming test in mice, oral administration of guarana extract (25 and 50 mg/kg) and caffeine (10 and 20 mg/kg) significantly decreased the duration of immobility, compared with control group (Campos, 2005). In a study by Espinola (1997), oral administration of suspensions of guarana (45 and 450 mg/kg/day) to mice increased their swimming capacities in the forced swimming test after 100 and 200 days of treatment but it was only statistically significant at 45 mg/kg/day. In addition, the known scopolamine amnesic effect was blocked by acute administration of 1 mg/kg i.p. caffeine or 3 and 30 mg/kg i.p. guarana in mice as well as by administration of approximately 33 mg/kg/day guarana in rats. The amnesic effects of scopolamine were also counteracted by oral administration of 30 mg/kg of a crude lyophilised extract of *Paullinia cupana* (var. *sorbilis* [Mart.] Ducke).

Effects on the CVS (bibliographic data):

Quercetin: The cardioprotective activity of quercetin in patients is shown in studies performed on cellular and animal models. The role of quercetin in preventing cardiovascular diseases has been largely associated with its anti-inflammatory and antioxidant properties (Russo, 2012).

Caffein and theobromine: Theobromine and its derivatives act as cardiac stimulants (Windholz, 1983; Bonati, 1984).

2.5.2.4. Pharmacodynamic drug interactions

No specific drug interaction studies were performed, and literature review did not identify any pharmacodynamic interaction related to the components of the finished product.

2.5.3. Pharmacokinetics

Three product-specific studies conducted by the applicant are presented in addition to some selected published data for the marker substances (quercetin, caffein and theobromine):

- A toxicokinetic evaluation of quercetin, caffeine and theobromine in Göttingen Minipigs after 28-day dermal administration of LH-8 cutaneous solution.
- A toxicokinetic evaluation after oral administration of LH-8 cutaneous solution to rats (Naravaneni R, Report N° 56191, 2023).

- An *in vitro* percutaneous absorption study in human skin evaluating the absorption and skin distribution of quercetin contained in CG 210 (ProviSkin, 2017).

Absorption

Studies conducted by the applicant

Toxicokinetic evaluation after dermal administration of LH-8 cutaneous solution: A 28-day dermal toxicity study in Göttingen Minipigs, including a toxicokinetic assessment of quercetin, caffeine and theobromine. This study was conducted with LH-8 cutaneous solutions at 3 concentrations:

- the lowest concentration corresponded to 11.125% of liquid ethanolic extract of Allium cepa *and Citrus limon* + 0.055% of dry extract of *Paullinia cupana* + 0.055% of dry extract of *Theobroma cacao*;
- the mid concentration was the intended clinical LH-8 cutaneous solution, corresponding to 22.25% of liquid ethanolic extract of *Allium cepa* and *Citrus limon* + 0.11% of dry extract of *Paullinia cupana* + 0.11% of dry extract of *Theobroma cacao*;
- the highest concentration corresponded to 44.50% of liquid ethanolic extract of *Allium cepa* and *Citrus limon* + 0.22% of dry extract of *Paullinia cupana* + 0.22% of dry extract of *Theobroma cacao.*

This dermal study was conducted under maximalised conditions, with a dose volume administered to each animal of 2 mL/kg (1 mL/kg/site) applied to both flanks, on a treatment site representing at least 10% of body surface area in total. The time of contact with the test item was approximately 20 hours. LH-8 cutaneous solutions were well tolerated at all the concentrations tested, with no noteworthy local or systemic effects.

Toxicokinetic evaluation after oral administration of LH-8 cutaneous solution: A toxicokinetic evaluation after oral administration of LH-8 cutaneous solution to rats was included the *in vivo* Rat Micronucleus Test (Naravaneni R, 2023), conducted by the applicant and is discussed in the toxicology section.

In vitro human skin permeability of quercetin: To evaluate the percutaneous absorption and skin distribution of quercetin contained either in CG 210 (LH-8) or in CG 428 cutaneous solution, an *in vitro* percutaneous study in human skin was conducted (ProviSkin, 2017). Quercetin's permeation following either a single application, or two applications during a 24h-period was evaluated. Each dose was applied over the external skin surface over the skin samples mounted in Franz Cells. One or two applications *per* day were performed on the skin for 24 hours to measure diffusion kinetics. Samples from the receptor fluid, with sink conditions, were collected after 2, 4, 8, 12, 18 and 24 hours. At the end, the amount of quercetin that remained on the skin surface was removed by washing, the cells were dismantled, and the different skin layers were separated to determine the amount of quercetin contained in each skin layer. Measurement of percutaneous absorption and assays of quercetin in the various samples obtained were performed by a HPLC analytical method (validation report not submitted). According to the applicant, the main conclusions were:

- The amount of quercetin which penetrates in skin strata was therapeutically relevant when considering the hair bulb as the target site located in the superficial skin layer.
- The percentage distribution of quercetin through the various skin strata was found very similar irrespective of the concentration or the number of applications.
- Increasing the volume of the applied products on the scalp surface produced some detectable increase of quercetin accumulation in the skin strata, albeit that doubling quercetin concentration did not result in a proportional permeation, confirming the presence of a saturation effect for quercetin skin absorption, as already reported in the literature.

- Since no quercetin could be detected in the bottom receptor fluid of the Franz cell set-up, this strongly suggested that no significant blood penetration should be expected, and this for both formulations applied either once or twice daily.

Bibliographic data

Quercetin: Alonso (2014) and Vicentini (2008) studied the in vitro percutaneous penetration of quercetin through excised pig skin biopsies placed on Franz static diffusion cells. A high amount of quercetin was recovered from the surface of the skin. Application of quercetin dissolved in ethanol applied to pig skin in Franz static diffusion cells resulted in 3.14 % of administered dose detected in epidermis and dermis (Alonso 2014). The total percutaneous absorption of quercetin was very low.

Caffein and theobromine: The *in vitro* transdermal delivery of saturated solutions of guarana extract in water was determined using Franz-type diffusion cells containing full thickness porcine ear skin. The greatest steady state flux of caffeine and theobromine were 19µg/cm²/h and 0.45 µg/cm²/h, respectively (Heard, 2006). The follicular penetration of caffeine dissolved in Dulbecco's phosphate-buffered saline was studied in human skin (Traur, 2010). The skin samples had a high, low or no follicular density. DPBS was used as the receptor medium of the Franz diffusion cells. Samples taken from the receptor chamber were examined using HPLC. After 24 hours, the skin sample that had a normal follicular density had the lowest absorption, and the high follicular density sample had the highest percentage of absorption.

Caffeine solution 4 mg/mL was applied to mounted human skin and six hours after application, skin samples were cleaned with soap and isotonic water. Permeation was measured for 42 hours. Caffeine permeation was reported to be 24 % in cells that were not washed, and 8% in cells that were washed (Luo, 2015).

Studies were done to examine the effect of human skin thickness and occlusion on the absorption of caffeine (Treffel, 1992; Wilkinson et al., 2006). Occlusion did not have an effect. The amount of caffeine in the skin membrane was not affected by skin thickness. After topical administration of radiolabeled caffeine in ethanol (1 mg/mL, [1-methyl-14C] caffeine) to female weanling Yorkshire swine percutaneous absorption was determined from total urine (5.8 ± 0.4 %) and faeces excretion (4.2 ± 1.0 %) of radiolabeled caffeine, to a total of 10.0 ± 1.3%. (Carver, 1989). Radiolabeled caffeine dissolved in ethanol was topically administered in female weanling Yorkshire type pigs. A non-occlusive protective patch was placed around the application area for 48h. The animals were sacrificed when radioactivity levels in urine reached background levels. Percutaneous absorption was determined from urine (14 ± 3 %) and faeces excretion (6 ±6 %) of dosed radiolabeled caffeine to a total of 20.0 ± 9%. (Reifenrath, 1984).

According to the applicant there is no information regarding percutaneous absorption of theobromine. However, it is noted that since theobromine is structurally close to caffeine, similar skin permeation parameters could be expected.

Distribution

Conventional pharmacokinetic studies to evaluate the distribution parameters of the product were not conducted by the applicant. No data were identified in the literature. According to the applicant, the low dose applied of quercetin (0.0925 mg/day), caffeine (0.21 mg/day) and theobromine (0.127 mg/day) *via* the therapeutic dose of the product and the very low skin absorption of quercetin, tissue distribution can be considered negligible, and no safety concern is expected in terms of quercetin distribution in the body.

Metabolism

Metabolism of quercetin, caffeine and theobromine was described in the literature (Harwood, 2007; Arnaud, 2011). However, due to the very low applied dose of the active constituents, their route of administration not involving the first-pass hepatic metabolism and the low transcutaneous passage for some of them, no toxicological concern is expected in experimental animals and humans. Caffeine is not metabolised in detectable amounts during percutaneous absorption (Bronaugh, 1989).

Excretion

Published data indicate that quercetin, caffeine and theobromine are mainly excreted into urine after oral administration (Harwood, 2007; Arnaud, 2011). No information is available on the excretion of LH-8 cutaneous solution, quercetin, caffeine, or theobromine into milk, after skin application. However, due to the low transcutaneous passage of quercetin, and the low applied dose of the active constituents, no toxicological concern is expected from the milk excretion of these substances.

Pharmacokinetic drug interactions

The product is intended to be applied on the skin and the applicant did not carry out additional studies.

2.5.4. Toxicology

The applicant submitted literature data and conducted a 7-day toxicity study in rats (oral administration), a 28-day toxicity study in mini pigs (dermal administration), and local tolerance tests. No carcinogenicity studies, or reproductive and developmental toxicity studies were conducted. *In vitro* and *in vivo* genotoxicity tests were performed but the comparability of the tested batches to the product of the present application is unresolved. In response to the CHMP, the applicant has performed a new ames test with cocoa dry extract, which showed no mutagenic potential.

2.5.4.1. Single dose toxicity

Bibliographic data, mainly on oral administration, was presented for *Paullinia cupana* and *Citrus limon*, and the three compounds quercetin, caffeine, and theobromine. The only endpoint discussed was the lethal dose, LD_{50}, which has no regulatory value. Despite the lack of information on dermal use of large amounts, the well-known character of the herbal substances from the food sector contributes to the overall assessment. Further studies on single dose toxicity are not required.

As the finished product contains 16.5% ethanol, the applicant was requested to include all recommended warnings in the SmPC and further evaluate the potential risks of systemic exposure in the most vulnerable patient categories. The transdermal passage of ethanol after administration of the product to the skin has not been analysed in any of the performed *in vivo* animal studies or the clinical study, and the safety assessment was consequently performed as hypothetical calculations based on literature data. The calculations provided thus far provide no safety margins to blood alcohol concentrations known to cause CNS effects in small children. Under occlusive conditions (administration site covered after administration), it is estimated that the resulting blood alcohol concentration in the youngest intended patient category is approximately 7 times above the known effect level, and in non-occlusive conditions 2.9 times below the known effect level. These calculations are based on single dose administrations and do not take into account that the product is applied twice daily during several months.

2.5.4.2. Repeat dose toxicity

No dermal, long-term, repeat-dose studies have been performed. The majority of the submitted information regarding repeat-dose toxicity is based on oral administration, mostly from bibliographic sources. The applicant conducted two repeat-dose studies, one in rats (oral route) for 7 days and one in mini-pigs (dermal administration) for 28 days.

The applicant suggested that the product is used for at least 3 months with a considerably longer anticipated duration of use in clinical practice. The submitted repeat-dose studies cover 4 weeks of administration. The applicant is planning to conduct two studies post approval (a 39-weeks dermal toxicity study in mini-pigs; a 26-weeks oral toxicity in adult rats), both including toxicokinetic and full histopathology, but this is not acceptable in a *post* approval setting and is not in line with CHMP Guideline on Repeated Dose Toxicity (CPMP/SWP/1042/99 Rev 1). It is emphasised that the value of oral toxicity studies is very limited.

The lack of information regarding repeat-dose toxicity after dermal administration is not acceptable. Without further information, the safe use of the product for the suggested duration of use, was not demonstrated. If sufficient published information cannot be found in the public domain, product specific, repeat-dose toxicity studies are required (as per the above-mentioned Guideline), focused on the effects seen after long-term dermal administration of the product of this application. The studies should also include toxicokinetic evaluations of all relevant constituents. This remains an unresolved issue, even after the submission of applicant's response provided during assessment.

The applicant did not sufficiently address the toxicokinetics after dermal administration of this product. The kinetics from the 28 days-study in minipigs indicate that the uptake of compounds is low but not negligible. It is also noted that only caffeine, theobromine and quercetin are monitored. The kinetics of the other compounds of the product are still unknown, including the systemic uptake of the excipient, ethanol.

The absorption and systemic exposure of the different components of the extracts after dermal application should generally be described in detail in an application. Without this information, systemic effects after dermal application cannot be excluded and all effects seen after repeated oral administration must be regarded as potential adverse effects of the product. The lack of systemic exposure is not established through measured plasma concentrations in animals or humans.

The first assessment was based on the applicant's assumption, that the systemic exposure is close to nil. The applicant did not show that, in mini pigs, the uptake of caffeine and theobromine is low but not negligible. No information was provided regarding the transdermal passage of other constituents of the product or of their pharmacological and/or toxicological effect.

Bibliographic data

***Allium cepa L.* (Onion)/quercetin**

Study ID/ Reference	Species/ Sex/ Number/ Group	Test item/ Dose/Route	Duration	NOEL/ NOAEL (mg/kg/ day)
Ruiz, 2006 (file named Ruiz 2009 in dossier)	Mouse/ M+F/ N=5	Quercetin 0, 30, 300, 3000 mg/kg/day **Oral** in diet	28 days	

Major findings: Significant changes in hematological parameters (Increase in RBC and hematocrit levels). Small increases in organ weight not accompanied by changes in clinical chemistry or histopathology. Decrease in serum cholesterol levels.

Barrenetxe, 2006	Mouse	Quercetin 25 mg/day **Oral** in diet	28 days
Major findings: Effects on small intestine enzymatic activity and nutrient absorption, although the morphology of the jejunum was not altered. No effect on organ weight or body weight.			
Azuma, 2010	Rat/male/	Allium cepa 19, 34, 47 and 94 mg/kg /day of quercetin aglycone *Or* Quercetin 2, 63, 95, 126, 157, 315, 630, and 1260 mg/kg body weight/day **Oral** in diet	4 weeks
Major findings: Significant increases in the ratios of the liver and kidney weights to the body weight at highest doses.			
Nakamura, 2000	Rat/male	Quercetin Up to 1000 mg/kg/day **Oral**	22 days
Major findings: No significant effects on food intake, body weight or relative and absolute liver weight.			
NTP, 1992	Rat/M+F/50	Quercetin 0, 1000, 10 000, 40 000 ppm corresponding to 40, 400 and 1900 mg/kg/Day **Oral** in feed	104 weeks
Major findings: Reduced body weight gain in male and female rats receiving 40,000 ppm Kidney male rat: dose-related increases in the severity of chronic nephropathy and a slight increased incidence in focal hyperplasia of the renal tubule epithelium. Parathyroid hyperplasia, indicative of renal secondary hyperparathyroidism, also increased incidence in dosed male rats. No apparent effect of quercetin on the kidney of female rats. Renal tubule adenomas and adenocarcinomas in male highest dose group. None in controls. Hyperplasia and adenomas in all dose groups (hyperplasia: 2/50, 2/50, 6/50, 8/50; adenoma: 1/50, 2/50, 7/50, 6/50). The overall incidence of renal tubule adenoma or adenocarcinoma combined in male rats was 1/50 in controls and 9/50 in the high- dose group			

***Paullinia cupana Kunth.* (Guarana)/caffeine**

Study ID/ Reference	Species/ Sex/ Number/ Group	Test item/ Dose/Route	Duration	NOEL/ NOAEL (mg/kg/ day)
Mattei, 1998	Male swiss mice, and male wistar rats	Guarana seed powder 3 mg/ml **oral** in drinking water, (corresponding to ca 262 mg/kg/day)	7 months	
Major findings: No alterations of the heart, lungs, stomach, small and large intestine, liver, pancreas, kidneys, bladder and spleen.				
OECD SIDS 2002	B6C3F1 mice M+F	Caffeine about 21, 44, 85, 130, 167 mg caffeine/kg/day for males and 25, 47, 88, 134, 180 mg caffeine/kg/day for females **Oral** in drinking water	90 days	The highest tested dose: male 167 mg/kg/day; female 180 mg/kg/day
Major findings: Mean body weight gain significantly, but not dose-dependently, depressed in middle male dose groups (44 to 130 mg/kg/day) while females showed only little variation. Water consumption was decreased at 130/134 and 167/180 mg/kg/day but increased in the other groups. Mild adaptive changes in the salivary glands at 167/180 mg/kg/day.				

IARC, 1991 (referred as IARC 1992 in NCO)	Female albino rats	**Intragastric cannula**	100 days	

Major findings: Above 110 mg/kg/day hypertrophy of the adrenal cortex and atrophy of the thymus gland. psychotic-like mutilation, gastric ulcers, hypertrophy of the salivary glands, liver, heart, kidneys and lungs, inhibition of oogenesis, minor changes in organ water levels, and an occasional death apparently from bronchopneumonia. Although no major change in growth rates or eating and drinking habits was apparent, some polydipsia and diuresis, thyroiditis, occasional dermatitis, some degree of nephritis, and loss of red pulp in the spleen were seen. The sensitivity of rats to the lethal effects of caffeine increased with age; caffeine was more toxic in male than in female rats.

Antonelli-Ushirobira, 2010	Wistar rats M+F	Semipurified extract (EPA fraction, containing caffeine) of *Paullinia cupana* seeds 30, 150 or 300 mg/kg/day **Oral** in feed	90 days	

Major findings

The rats treated with 150 or 300 mg/kg/day gained weight more slowly and lost kidney weight. Possible greater biological susceptibility of males. No toxicity at the smallest dose evaluated (30 mg/kg/day). Blood leukocytes significantly reduced in both males and females at 150 mg/kg and in males only at 300 mg/kg. The males showed increases levels of alkaline phosphatase and glutamic-pyruvic transaminase (GPT) in all treated groups. In females, only those treated with 150 mg/kg showed changes in the levels of alkaline phosphatase, which increased in relation to the control group. All the groups of treated females showed increased levels of urea, glycemia, and decreased triglycerides. The females treated with 150 mg/kg and 300 mg/kg showed increased levels of amylase.

OECD SIDS, 2002	Fisher 344 rats	Caffeine Males: About 19.7, 42, 85.4, 151, 272 mg/kg/day Females: About 23, 51, 104, 174, and 287 mg/kg/day **Oral** in drinking water	90-day	151(male)/ 174(female) mg/kg/day.

Major findings: At 272/287 mg/kg/day, the body weight gains and water consumption were significantly decreased. Up to151/174 mg/kg/day, no clinical signs of toxicity and no dose-related changes in clinical chemistry were observed. No significant changes in gross morphology or microscopic findings were observed except for a dose-dependent cellular enlargement in salivary gland, which is a well-known pharmacological effect of caffeine (sympathicomimetic). These morphological changes are not considered to be an adverse effect of caffeine. Finally, microscopic evaluation of sex organs revealed no significant differences between exposed and control rats.

***Theobroma cacao* L. (Cocoa)/theobromine**

Study ID/ Reference	**Species/ Sex/ Number/ Group**	**Test item/ Dose/Route**	**Duration**	**NOEL/ NOAEL (mg/kg/ day)**
Tarka, 1979	Sprague-Dawley rats M+F	Theobromine 0.2% to 1%, (corresponding to 90 – 500 mg/kg/day in males and 140 – 600 mg/kg/day in females) **Oral** in feed	4 weeks	

Major findings: Anorexia (except in female rats given the 22 % casein diet), decreases in body weight in mature rats, growth retardation in immature rats and atrophy of the thymus glands in rats of both sexes and testicular atrophy in male rats.

Gans, 1984	Sprague-Dawley rats male	Theobromine 0.8%, corresponding to 800 mg/kg/day in male rats **Oral** in feed	7 weeks	

Major findings: Decreased food consumption and body weight gain. Decreased thymus weight and testicular atrophy with spermatogenic cell degeneration and necrosis.

Tarka, 1983 (Ref not submitted, ref to IARC 1991)	Sprague-Dawley rats M+F	**Theobromine** 0.02%, 0.1% and 0.2%, (corresponding to 25, 125 and 250 mg/kg/day) **Oral** in feed	90 days
Major findings: Reduction in body weight gain and testicular weight in males at 250 mg/kg/day.			
Gans, 1980	Mongrel dogs male	Theobromine 75-150mg/kg/day, (Gans, 1980). Or 25-150mg/kg/day, (Gans, 1980). **Oral**	21-28 days Or 1 year
Major findings: Degenerative, fibrotic cardiomyopathy limited to the right atrial appendage of the heart reported but no testicular nor thymus atrophy. A clear relationship between dose, theobromine plasma concentration and severity of the atrial lesion could not be established. The authors considered that the theobromine associated right atrial cardiomyopathy probably represents a specific response of the dog to vasodilator drugs.			

2.5.4.3. Genotoxicity

The applicant performed a standard battery of genotoxicity tests on LH-8 or its constituents. The battery included *in vitro* tests (Ames and CHO) with the 3 components of LH-8 (Cocoa Dry Extract, Guarana Dry Extract and Liquid Ethanolic Extract of *Allium cepa* and *Citrus limon*) as well as an *in vivo* rat micronucleus test with LH-8. The tests did not indicate any genotoxic potential for LH-8 or its constituents but the comparability of the tested batches to the product of the present application was questioned. In response, the applicant has performed a new Ames test with cocoa dry extract, which showed no mutagenic potential. Ames tests on the Guarana Dry Extract and Liquid Ethanolic Extract of *Allium cepa* and *Citrus limon* have not been submitted. As there are positive genotoxicity test reported in then literature for several of the constituents of the product, product specific genotoxicity tests were required to establish the lack of genotoxic potential of this product.

2.5.4.4. Carcinogenicity

No carcinogenicity study was conducted with LH-8 cutaneous solution. According to ICH S1A, carcinogenicity studies should be performed for any pharmaceutical whose expected clinical use is continuous for at least 6 months. It should be noted that the recently (Aug 2022) published part II of the ICH S1B(R1) step 4, presents an alternative option, where a weight of evidence (WoE) approach may be applied to investigate whether or not a 2-year rat study is likely to add value to a human carcinogenicity risk assessment.

The published literature presented by the applicant is not consistent regarding the genotoxic and/or carcinogenic potential of the constituents of the product, i.e., ethanol, quercetin, caffein, guarana etc. There are negative reports as well as numerous publications showing positive results. It was not specified how the cited references were chosen, what filters, inclusion or exclusion criteria were used, etc. The information chosen from each reference seems arbitrary and selected. Based on these, pre-chosen references, the applicant concludes that there is no suspicion for a carcinogenic potential of LH-8 cutaneous lotion based on:

- the lack of carcinogenic effects reported by the oral route in rats,
- the absence of mutagenic properties for LH-8 and its constituents,
- the low daily dose level of LH-8 cutaneous lotion applied on the scalp and

- the absence of adverse cutaneous reaction such as hyperplasia after repeat-dose application in humans.

Additionally, the applicant refers to the good local tolerance of LH-8 cutaneous solution in human, supported by its extensive use as a cosmetic hair lotion (CG 210) at the same dose levels, without any adverse effects reported for the consumers as mentioned in the cosmetic safety report data.

There are, however, several inconsistencies in these statements:

- Carcinogenic effects are reported by the oral route in rats but have been considered probably not relevant for humans.
- Several constituents of the product have shown mutagenic properties in different test systems, and there are unresolved issues regarding the product specific genotoxicity tests.
- The statement that repeat-dose application in humans does not result in hyperplasia or other adverse cutaneous reactions is not supported by histopathologically assessed biopsies or other evidence. If not specifically studied, the different alterations to the skin are not easily classified.
- In the clinical parts of the application, it is emphasised that the product stimulates cell proliferation, as evidenced by increased levels of the proliferation marker Ki67 in biopsies from individuals treated with CG210 (Cucé 2011 and 2012).
- In contradiction to the statement of the applicant, adverse reactions are reported after the cosmetic use of the product, but as the use is always accompanied by other drugs it cannot be established whether they can be attributed to LH-8 or the other products.
- Listed adverse reactions in section 4.8 of the SmPC are eczema (common), skin irritation (common), scalp folliculitis (uncommon) and acne (uncommon).

This product is intended for long term application to the skin. All local reactions and potential irritant properties must hence be accounted for when considering the carcinogenic potential of the product.

To use the weight of evidence approach, robust, supportive data should have been presented from both, genotoxicity and long-term repeat-dose tests on cutaneous use in relevant species. The WoE approach is not acceptable. The lack of carcinogenic potential was not established for this product. Carcinogenicity tests in accordance with ICH S1B(R1) step 4 are missing and still required.

2.5.4.5. Reproductive and developmental toxicity

No reproductive studies were performed. No published/bibliographic studies were submitted regarding reproductive and development toxicity of the product or the herbal extracts. The applicant focused on published information regarding the three compounds quercetin, caffeine, and theobromine. Several effects are seen in the referred literature, but the applicant claims that all observations of reproductive or developmental toxicity are reported at high doses "far in excess" of the daily dose applied on the scalp *via* LH-8 cutaneous solution at the therapeutic dose level" without further evaluation or comparison. Such a statement is to be supported by calculations of the comparable dose in humans, and comparisons to the established plasma concentrations after administration of the product in question. The applicant has not provided any evidence in support of the statement.

The lack of systemic exposure is not sufficiently demonstrated through measured plasma concentrations in animals or humans. Further information has been requested regarding the systemic exposure to constituents

present in relevant amounts. The toxicological assessment cannot be fully concluded until those issues are resolved.

In the absence of sufficient data, the applicant has suggested that the use of the finished product LH-8 cutaneous solution during pregnancy and lactation is not recommended. This is reflected in the SmPC.

2.5.4.6. Toxicokinetic data

The applicant conducted a 28-days dermal toxicity in mini-pigs, including toxicokinetics. The kinetics from the 28 days-study in minipigs indicate that the uptake of compounds is low, but not negligible. Only caffeine, theobromine and quercetin are measured. The systemic exposure to the other compounds is still unknown.

2.5.4.7. Local Tolerance

The applicant conducted studies related to local tolerance:

- *In vitro* ocular irritation test on NCTC L929 cell line;
- *In vivo* acute eye irritation study in rabbits;
- *In vitro* skin irritation assay on human reconstructed epidermis.

Available published non-clinical data has also been presented regarding local tolerance and phototoxicity of LH-8 and/or its components. The studies regarding ocular tolerance and local dermal tolerance give no cause for concern.

In the *in vitro* skin irritation study on human reconstructed epidermis (SkinEthic™ model), the main herbal extract component of LH-8 (liquid ethanolic extract of *Allium cepa fresh bulb and Citrus limon fresh fruit*) was evaluated for skin irritation potential in an *in vitro* human reconstructed epidermis. Under the experimental conditions in this study, the test item was considered to be non-irritant to the skin.

2.5.4.8. Other toxicity studies

Studies on impurities: The applicant performed a risk evaluation regarding nitrosamines, but further information is pending. Tests in compliance with the Ph.Eur. requirements have been performed regarding pesticides residues, heavy metals, radioactivity, mycotoxins and microbiological quality, see quality section.

Regarding degradants, the applicant stated that the herbal substances are generally recognised as safe for oral consumption and that the degradation products are well documented in the literature not to be toxic.

Erythrocyte Osmotic fragility: The applicant submitted information that oral intake of onion was reported to cause haemolytic anaemia in rats and dogs. This was likely related to the presence of peroxidizable polyunsaturated fatty acids and malonyl dialdehyde, that alter the stability of the erythrocyte membrane. Further testing in rats, *in vivo* and *in vitro*, concluded that onion increases osmotic fragility of red blood cells.

The applicant emphasised that the product was well tolerated in the 28-days dermal study in mini-pigs, and that the evaluation of haematology, coagulation and clinical chemistry parameters did not indicate any adverse effects or signs indicative of haemolysis. No local effects, indicating this type of effect, were seen at the site of administration or the histopathologically examined surrounding tissue. Based on the provided

information, it is concluded that the risk for haemolysis caused by the potential content of peroxidizable polyunsaturated fatty acids and malonyl dialdehyde is very low.

2.5.5. Ecotoxicity/environmental risk assessment

The applicant submitted a brief ERA. As the active substances are natural substances, the use of which will not alter the concentration or distribution of the substance in the environment, the product is not expected to pose a risk to the environment.

2.5.6. Discussion on non-clinical aspects

The non-clinical documentation was based mainly on bibliographical review of the well-known herbal substances which are part of LH-8. Literature data on three markers of the extracts: quercetin, caffein and theobromine, were also included. The applicant performed some studies of their own.

Herbal extracts in general contain a multitude of active compounds. Despite this, the applicant has chosen to present and assess only a few components or "markers" that they consider responsible for the claimed efficacy. A detailed assessment of the complete content of the product has been initiated but further information not provided.

As the four herbal substances are well-known from the food sector, the effects from oral administration are well described in the published literature. This has been acknowledged, and to some extent utilised by the applicant. In the areas where published literature is quoted, the applicant has not summarised the scientific knowledge but rather selected a few studies without explaining or discussing the reason for the choice. It should be noted that, for a new marketing authorisation application under Article 8.3, waiving certain studies or replacing original studies by literature data is possible, as long as an acceptable justification is provided. The applicant has however not compiled a complete dossier regarding the dermal application of these extracts, nor fully justified the lack of certain data.

Pharmacology: No proper proof of concept study for the claimed mechanisms of action was presented. Animal models relevant for the proposed indication are available but are not discussed. Nor has a clear relation between dose and activity been provided. The only non-clinical efficacy studies presented for a solution allegedly equivalent with the product, are the *in vitro* studies. During the pre-submission interactions, it was raised that *in vitro* inflammation data may not be seen as a sufficient proof of concept. A proof of preliminary efficacy in an *in vivo* model is normally needed to confirm the *in vitro* findings. The applicant argues that, as the ingredients are known to be safe in humans, no animal tests are required before tests in humans and that human data obtained on androgenetic alopecia (AGA) can serve as a proof-of-concept of AA evaluation. Regarding safety, these arguments are agreed, however, it is not agreed that data obtained on AGA can serve as a proof-of-concept for a potential effect in AA. The mechanisms causing hair loss are not fully elucidated for any of the two alopecia variants, while there are significant differences.

There is also a lack of pre-clinical data demonstrating the contribution of each of the extracts to the claimed effect or as support for the relative content of the extracts used in the product. The plants in the composition of the active substance are reported to have different anti-inflammatory and antioxidant effects. The applicant claims that the multiple substances contained in the four plants in LH-8 formulation allow for a pleiotropic effect on multiple targets, simultaneously, however, has not provided a fully acceptable justification for lack of *in vivo* preclinical proof of concept tests or for the composition of the product. With the limited effect seen in clinical setting, the CHMP does not consider relevant to pursue this issue further.

Furthermore, there are questions regarding the relevance and performance of the *in vitro* methods. The use of HUVEC cell line is unclear as it is not derived from dermal vascular endothelial cells. The presented discussion on the relevance of the studied mechanisms is mainly hypothetical, with some details supported by references. It is plausible that AA is caused by a collapse of the relative immune privilege (IP), which protects the hair follicle. The endothelial cells that line the luminal site of blood vessels do express a range of proinflammatory adhesion molecule which may attract and direct patrolling immune cells to extravasate from the vasculature into underlying tissue, supposedly causing inflammation and immune privilege collapse. However, there is no direct relation demonstrated between improvement of AA and the specific molecules selected to study. Some effects were detected in the *in vitro* studies, but at high concentrations only. Reference is made to a few publications regarding the effects of three indicated marker-substances, although the product contains several other substances for which no information was presented. Overall, the relevance of effects studied in the presented literature, for the claimed clinical effect of the product, is not clear. It is not likely that the mechanism of action will be completely clarified, and the issue is therefore not further pursued by the CHMP.

Based on the results of an *in vitro* penetration study in human skin, the applicant claims that the amount of quercetin which penetrates in skin strata is therapeutically relevant, although no significant efficacy data demonstrating effect at such low concentrations are presented. No skin penetration data for caffein and theobromine were analysed, which would have been valuable for demonstrating that pharmacologically active concentrations have reached the target. Regarding *in vivo* exposure of caffein and theobromine after dermal application, the applicant did not discuss the results in relation to concentrations for which an anti-inflammatory or an anti-apoptotic effect has been shown. The exposure measured in the mini-pig study was compared to concentrations of caffein and theobromine producing effect *in vitro*. Such comparisons are not completely valid for assessment of a possible pharmacological effect, as the exposure at the hair-bulb may be different from blood exposure. Nevertheless, the comparison shows that plasma exposure in minipigs after dermal application with a dose several fold above, or more, above the clinical dose is 12% for caffein and 5% for theobromine of the lowest concentration required for an *in vitro* effect. As it is unlikely that the concentration at target (in vascular endothelial cells surrounding the hair bulb), is much higher than in plasma, it appears that the data presented for *in vivo* exposure of caffeine and theobromine does not support an effect of suggested clinical dose.

Data presented as secondary pharmacodynamics are only bibliographic and refers to antioxidant activity, effects on the CNS and the cardiovascular system. In view of the route of administration, cardiovascular effects reported after systemic administration of caffeine or theobromine are not considered to be very relevant. A standard battery of safety pharmacology studies is not expected as safety concerns can be addressed as part of the dermal, repeated-dose toxicity study. No product-specific safety pharmacology studies or pharmacodynamic interaction studies have been submitted. A very limited number of bibliographic studies have been presented.

Pharmacokinetics: Product-specific *in vivo* exposure data after topical administration are available only from one study conducted by the applicant: toxicokinetic evaluation after 28-days dermal application in mini-pig. In addition, the applicant presents a toxicokinetic evaluation after oral administration of LH-8 to rats and an *in vitro* percutaneous absorption study in human skin of quercetin contained in CG 210. There is no justification for the choice of substances which have been analysed *in vivo* and *in vitro*. Caffeine and theobromine were analysed in the *in vivo* studies but not in the in vitro percutaneous absorption-study. The highest measured exposures in minipig, as C_{max}, were after 900 mg/kg/day, on day 28, 171 ng/mL and 33.1 ng/mL of caffein and theobromine, respectively. The dose corresponds to a human equivalent dose (HED) of

851 mg/kg/day, which is at several fold above clinical dose. Analysis of quercetin showed no detectable exposure *in vivo* after dermal administration of LH-8 in ether of the TK studies (LoQ: 0.50 ng/mL).

The applicant did not conduct any conventional pharmacokinetic studies to evaluate tissue distribution, metabolism, excretion, or pharmacokinetic drug interactions. Limited bibliographic data for the claimed main active substances (quercetin, caffeine and theobromine) were submitted. Cinainu also contains a high amount of ethanol. The transcutaneous passage of ethanol after application of LH-8 solution to paediatric scalp skin and the potential for induction of systemic effects has not been adequately addressed. This is especially important for the youngest paediatric population.

Toxicology: To account for the toxicology of Cinainu, the applicant has submitted published literature of the different herbal substances included in the composition. In addition, the applicant has conducted a 7-day toxicity study in rats (oral administration), a 28-day toxicity study in mini pigs (dermal administration), as well as local tolerance studies with the product of this application. No carcinogenicity studies, or reproductive and developmental toxicity studies were conducted with Cinainu, or the extracts thereof. Regarding genotoxicity, *in vitro* and *in vivo* genotoxicity tests were performed by the applicant but the comparability of the tested batches to the product of the present application is questioned. In response, the applicant performed a new Ames test with cocoa dry extract, which showed no mutagenic potential. Ames tests on the Guarana Dry Extract and Liquid Ethanolic Extract of *Allium cepa* and *Citrus limon* are pending.

The applicant states that "a bibliographic research" was performed, without further specification. As all four herbal substances, and all three chosen markers, are compounds of high scientific interest, a full literature search would be needed to render much more information than submitted by the applicant. In addition, the applicant's use of references is considered arbitrary. Several references are named wrong or cited incorrectly. The information given by the applicant is not always identical to what is stated in the reference, and several of the references provide more valuable information regarding the compounds of this application than what is included in the dossier.

Single dose toxicity: Bibliographic data, mainly on oral administration, was presented for *Paullinia cupana* and *Citrus limon*, and the three compounds quercetin, caffeine, and theobromine. The only endpoint discussed was the lethal dose, LD_{50}, which has no regulatory value. Despite the lack of information on dermal use, the well-known character of the herbal substances from the food sector contributes to the overall assessment. The acute toxicity may be regarded in light of the results from studies on repeat-dose toxicity. Further studies on single dose toxicity are not required.

Particular focus should be put on the potential systemic exposure to ethanol, which was not measured in the kinetic studies of the product, and the blood levels of ethanol after dermal administration of the product are unknown. The calculations submitted by the applicant show potential blood alcohol levels very close to or even above levels known to cause CNS effects in children. Under occlusive conditions (administration site covered after administration), it is estimated that the resulting blood alcohol concentration in the youngest intended patient category is approximately 7 times above the known effect level, and in non-occlusive conditions 2.9 times below the known effect level. These calculations are based on single dose administrations and do not take into account that the product is applied twice daily during several months.

Repeat-dose toxicity: No dermal, long-term, repeat-dose studies have been performed. The majority of the submitted information regarding repeat-dose toxicity is based on oral administration. One repeat-dose toxicity study has been performed in mini-pigs, covering 4 weeks of administration, but the applicant suggested that the product is used for at least 3 months with a considerably longer anticipated duration of use. In addition, the applicant suggested to conduct two studies post approval (39-weeks dermal toxicity

study in mini-pigs, including toxicokinetic and full histopathology; 26-weeks oral toxicity in adult rats, including toxicokinetic and full histopathology). It is not acceptable to perform these studies post approval, as per the valid CHMP guideline. In addition, it is emphasised that the value of oral toxicity studies is very limited for this application. The lack of information regarding repeat-dose toxicity after dermal administration is not acceptable. Without further information, the safe use of the product for the suggested duration of use, is not established.

The applicant insufficiently addressed the toxicokinetics after dermal administration of this product. The kinetics from the 28 days-study in minipigs indicate that the uptake of compounds is low but not negligible. Only caffeine, theobromine and quercetin are monitored. The kinetics of the other compounds of the product are still unknown, including the systemic uptake of the excipient, ethanol.

Genotoxicity: The applicant performed a standard battery of genotoxicity tests on LH-8 or its constituents. The battery included *in vitro* tests (Ames and CHO) with the 3 components of LH-8 (Cocoa Dry Extract, Guarana Dry Extract and Liquid Ethanolic Extract of *Allium cepa* and *Citrus limon*) as well as an *in vivo* rat micronucleus test with LH-8. The tests did not indicate any genotoxic potential for LH-8 or its constituents. The comparability of the tested batches to the product of the present application was questioned. In response, the applicant has performed a new Ames test with cocoa dry extract, which showed no mutagenic potential. Ames tests on the Guarana Dry Extract and Liquid Ethanolic Extract of *Allium cepa* and *Citrus limon* have not been submitted. As there are positive genotoxicity test in then literature for several of the constituents of the product, product specific genotoxicity tests would be required to demonstrate the lack of genotoxic potential of this product but are missing.

Carcinogenicity: No carcinogenicity study was conducted with the finished product LH-8 cutaneous solution. According to the ICH S1A guideline, carcinogenicity studies should be performed for any pharmaceutical whose expected clinical use is continuous for at least 6 months. It should however be noted that the recently (Aug 2022) published part II (addendum) of the ICH S1B(R1) step 4, presents an alternative option, where a weight of evidence (WoE) approach may be applied to investigate whether a 2-year rat study is likely to add value to a human carcinogenicity risk assessment. Nevertheless, the data presented by the applicant to waive the requirements for a carcinogenicity study are not sufficient, as the published literature is not consistent regarding the genotoxic and/or carcinogenic potential of the constituents of the product (quercetin, caffein, guarana, etc). There are negative reports as well as numerous publications showing positive results.

The applicant concludes that there is no suspicion for a carcinogenic potential of LH-8 cutaneous lotion based on the claimed lack of carcinogenic effects reported by the oral route in rats, absence of mutagenic properties for LH-8 and its constituents, low daily dose level of LH-8 cutaneous lotion applied on the scalp and absence of adverse cutaneous reaction such as hyperplasia after repeat-dose application in humans. Additionally, the applicant refers to the good local tolerance of LH-8 cutaneous solution in human, supported by its extensive use as a cosmetic hair lotion at the same dose levels, without any adverse effects reported for the consumers as mentioned in the cosmetic safety report data.

There are, however, several inconsistencies in these statements: carcinogenic effects are reported by the oral route in rats but probably have been considered not relevant for humans. Several constituents of the product have shown mutagenic properties in different tests. The statement that repeat-dose application in humans does not result in hyperplasia or other adverse cutaneous reactions is not supported by histopathologically assessed biopsies. If not specifically studied, the different alterations to the skin are not easily classified. In the clinical parts of the application, it is emphasised that the product stimulates cell proliferation. In contradiction to the applicant's statement, adverse reactions are reported after the cosmetic use of the product, but as the use is always accompanied by other medicines, it cannot be established whether they are

attributed to LH-8 or the other products. This product is intended for long term application to the skin. All local reactions and potential irritant properties must hence be accounted for when considering the carcinogenic potential of the product.

In order to use WoE, robust, in line with (Aug 2022) published part II (addendum) of the ICH S1B(R1) step 4, supportive data should be presented from both genotoxicity, and long-term repeat-dose studies on cutaneous use, in relevant species. The lack of carcinogenic potential has not been established for this product as no carcinogenicity tests in accordance with ICH S1B(R1) step 4 were conducted.

Reproductive and developmental toxicity: No reproductive studies were performed with LH-8 cutaneous solution. The applicant focused on published information regarding the three compounds quercetin, caffeine, and theobromine. Several effects are seen in the referred literature, but the applicant claims that all observations of reproductive or developmental toxicity are reported at high doses "far in excess" of the daily dose applied on the scalp via LH-8 cutaneous solution at the therapeutic dose level" without further evaluation or comparison. Such a statement should always be supported by calculations of the comparable dose in humans, and comparisons to the established plasma concentrations after administration of the product in question. The applicant has not provided any evidence in support of the statement.

Local tolerance: The applicant has conducted a few studies and in addition presents available published nonclinical data regarding local tolerance and phototoxicity of LH-8 and/or its components. Ocular tolerance and local dermal tolerance tests give no cause for concern.

ERA: The active substances are natural, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, the product is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

The applicant did not provide sufficient supportive information from a non-clinical perspective and major issues on the non-clinical aspects of the product have not been resolved. The safety of Cinainu for long-term use, in particular lack of carcinogenic potential and use in the young patient population, has not been sufficiently substantiated. The applicant has no long-term dermal toxicity studies, and very limited knowledge of the constituents of the product and their local effect or transdermal passage. The genotoxicity package is insufficient, no carcinogenicity study has been performed, and the submitted information if insufficient for a weight of evidence approach. Supporting information regarding the long-term safety cannot be easily derived from the RAAINBOW study, since comparability has not been established between the proposed medicinal product and the clinical batches that were used in the clinical RAAINBOW study.

In conclusion, the safety of the product for long-term use in the intended young patient population, in particular lack of carcinogenic potential, has not been sufficiently substantiated. The application is not approvable from a non-clinical point of view.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

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

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

Table 1. Tabular overview of clinical studies

Overview of the single pivotal RAAINBOW study in AA in children and adolescents.

Study ID/ Study date/ No. study centres and locations	Design	Study Posology	Study Objective	Subjs by arm entered / compl.	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
RAAINBOW study EudraCT 2016-003208-30 / ClinicalTrials.gov identifier NCT03240627 Study date: 01 Feb 2018 to 14 Sept 2022 (LPLV) 12 study centres (3 in Bulgaria, 2 in Germany, 1 in France and 6 in India)	Double-blind, randomised (2:1), multi-center study versus placebo	LH-8 or placebo applied on the whole scalp. Subjects aged 2 to 15 years: 0.882 ml (7 sprays); Subjects 15 to less than 18 years: 1.008 ml (8 sprays); To be applied twice daily on the whole scalp. 0.126 ml per spray	Efficacy and Tolerance of LH-8 cutaneous solution in children and adolescents with moderate to severe scalp alopecia areata (AA)	107 patients enrolled Evaluable 62 (42 LH-8 group and 20 placebo group)	6 months Treatment + 6 months Treatment-free period	M/F 45%/55 % 11 years old Average duration of disease since 1st episode 3 years	Children and adolescents (from 2 to less than 18 years) with active alopecia areata (AA) involving 25% to 95% of the scalp between 6 months and 3 years in duration	Relative change in scalp alopecia areata severity scores (SALT) from baseline value to be assessed after 24 weeks of treatment

The LH-8 cutaneous solution was developed as CG 210 for the treatment of androgenic alopecia for adults and is also currently being developed as CG 428 in Chemotherapy Induced Alopecia (CIA) at higher concentration. In support to the scientific rationale for evaluating LH-8 in AA, the applicant presents the results already obtained in humans with LH-8 AGA and CIA (including permanent chemotherapy-induced alopecia, pCIA). These were submitted to support the scientific rationale for evaluating LH-8 in AA, although both conditions are different in their pathophysiology.

Table 2. Several of the studies had an open design

Product	Population Name Design Duration Subjects N°	Title	Key findings
Studies in AGA			
LH-8	AGA ARIANE Controlled	Efficacy and Safety of a Topical Botanical in Female Androgenetic	Katoulis, 2018 increased hair density in the intervention

Product	Population Name Design Duration Subjects N°	Title	Key findings
	6 months 39 female	Alopecia: A Randomised, Single-Blinded, Vehicle-Controlled Study	group (great improvement in 7.7%, moderate in 88.5%, and stable in 3.8%)
LH-8	AGA CATSH 14 Comparative 2 months 240 female	Comparative Study of the Efficacy and Acceptability of a CG 210 versus a Food Supplement on Hair Loss, Hair Growth and Hair Pattern after 14, 28 and 56 days of use	Petitjean (Internal report, 2013) Women using CG 210 reported better and quicker outcomes than women using food supplement which contains 5-alpha-réductol (pumpkin seeds oil).
LH-8	AGA ACEPA 3 Open 3 months 19 male	Clinical Efficacy by Phototrichogram on Alopecia- male patients	Liu, 2013 The product was effective in preventing hair loss after 12 weeks of application.
LH-8	AGA Qilib-India Open 3 months 20 female 20 male	Safety and efficacy of a novel Botanical Hair Lotion in Indian men and women with androgenetic alopecia	Qilib India (Internal report 2016) Median improvement in scalp coverage (photo assessed by physician) at 3 months was 40% in males and 50% in females. in male, increase of hair density (macrophotography) + 13% at 1,5 month and +27% at 3 months (significant).
LH-8	AGA HIRUDOID Open 3 months 25 female 15 male	Assess product satisfaction of HIRUDOID (CG210) in male and female aged 25-45 y/o with hair loss	Hirudoid (Internal report 2021) After 2 months of use, 100% of respondent agreed that product helps reduce hair loss, they feel more hair volume, product helps feel that hair and scalp are healthy, and are overall satisfied.
Studies in CIA			
CG 428	pCIA VOLUME Controlled 6 months 35 female	Evaluation of the impact of a topical lotion on permanent chemotherapy induced hair disorders in cancer survivors	Kang 2020 Pilot, randomised, clinical trial, where the authors report an observed safety, tolerability, and a trend toward the efficacy of CG428 *vs* placebo
CG 428	pCIA VOLUME-2 Open 6 months 19 female	Evaluation of the impact of CG428 on permanent chemotherapy-induced hair and scalp disorders in cancer survivors, an open label 6 months extension of VOLUME	Volume 2 (Internal report 2018) Hair density and thickness were increased compared to baseline of VOLUME after 9 months of treatment with CG428 (respectively +28.00% and +31.54%). After 12 months of treatment with CG428, hair density and thickness were increased respectively by +26.01% and +17.58%.
CG 428	pCIA ALOBRCA Comparative 12 months 64 female	Trial of the efficacy and safety of CG428 lotion against the adjuvant chemotherapy induced alopecia in breast cancer patients.	Kojima, 2022 At the time of end of chemotherapy, all subjects had the grade 3 or 4 of hair loss. At the 6 months after the treatment, grade 0/1/≥2 was 50/38/12 (%) respectively. At the 12 months after treatment, grade 0/1/≥2 was 69/11/20 (%) respectively.
CG 428	pCIA RELAC	Assessment of the efficacy and safety of CG	Shiiba, 2015

Product	Population Name Design Duration Subjects N°	Title	Key findings
	Open 3 months 21 female	428 in the recovery from long term chemotherapy induced alopecia in women	Hair pattern improvement in 38.1%, 57.1% and 81% subjects after 1, 2 and 3 months respectively
CG 428	CIA CARA Open 3 months 10 female	Assessment of the Efficacy and safety of CG 428 in the Recovery from Chemotherapy Induced Alopecia in Women	Shiiba, 2015 Faster hair recovery confirmed by a reduction of baldness period in 100% of subjects. Reduction of baldness period from 21 weeks to an average of 9 weeks. No complete baldness observed during the study

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

No clinical plasma pharmacokinetic data have been presented. Since major constituents have already been identified and quantified in the LH-8 product, a worst-case scenario for their skin permeation into the bloodstream is proposed. It is based on the maximal blood concentrations that could be obtained with phytochemical entities of LH-8 that were well-identified and quantified by HPLC. The highest measured concentrations of these constituents that appeared in batches already produced were used. This estimation has been performed, A) for from both *Paullinia cupana* and *Theobroma cacao*, B) for from *Theobroma cacao*, C) for, and D) for from *Allium cepa*. In addition, the same process has also been done E) for 6 which was recently quantified as a marker of *Citrus lemon*.

Based on these results, even under non-occlusive conditions, as suggested for Cinainu, the risk is not negligible and should be further examined. In addition, the applicant has not discussed the significance of the product being applied twice daily during several months.

2.6.2.2. Pharmacodynamics

Mechanism of action

There is no dedicated study to support the mechanism of action. The applicant refers to a spectrum of anti-inflammatory and immune modulating effects based on *in vivo* and *in vitro* data, and literature. The mechanism of action for the combination of the herbal extracts is considered not fully characterised.

Primary and Secondary pharmacology

No dedicated pharmacodynamic studies were performed.

Table 3. The applicant refers to the perifollicular inflammation and the premature cell apoptosis studied *in vitro* and *in vivo*, and data from the literature

Study	Name/reference	Objectives	Main Outcomes
In vitro, HUVECs (Human Umbilical Vein Endothelial Cells)	TIE study / Bourguet, 2014	To demonstrate, at molecular level, pharmacological effects of LH-8 as anti-inflammatory agent	LH-8 was able to reduce
In human, scalp biopsies of 20 androgenic alopecia (AGA) patients, open-label	ACEBA 3 study / Cucé, 2011	To identify LH-8 PD characteristics that potentially allow LH-8 to exert beneficial functions or local effects on skin immunological defences and keratinocytes proliferation were studied	-Significant increase of BCL2+ cells in the epidermis, from 1.722% before treatment to 3.235% after treatment (+89%). -Significant increase of Langerhans CD1A+ cells in the epidermis, from 7.3% before treatment to 12.7% after treatment (+73.9%). -Significant increase by 41.66% of Ki-67 cells in the epidermis. -No statistical differences in the HSP47+ cells in the dermis.
In human, scalp biopsies of 25 healthy non-balding subjects	SCALP BCL2 study / Cucé, 2012	To determine the Bcl-2 level in the scalp of healthy non-alopecic subjects (to ensure safety of LH-8 at molecular level)	In the scalp of healthy subjects, the fraction of BCL2+ cells was in average 4.73%. The level of Bcl-2 in alopecic subjects' scalp after product application was restored to 3.235%, near but still lower than the normal level of 4.73%.
In human, 20 androgenic alopecia (AGA) patients, open-label	ACEPA 3 study / Casoli 2007	To find out if the afore-described pharmacological beneficial effects of LH-8 could be translated into clinical efficacy.	After 6 weeks of application, a substantial decrease of telogen hair (-8.20%), and increase of anagen hair (+2.31%), resulting in a significant increase of anagen / telogen ratio (+44.91%) – A/T ratio=4.30, above the normal level. After 12 weeks of product, the A/T ratio was maintained at 4.35; The number of new anagen hairs rose by 11.6%, thus improving density. Good safety of LH-8 observed.
In human, Efficacy and Safety of a Topical Botanical in Female Androgenetic Alopecia: A Randomised, Single-Blinded, Vehicle-Controlled Study	ARIANE study / Katoulis, 2018	To find out if the afore-described pharmacological beneficial effects of LH-8 could be translated into clinical efficacy	An increased hair density in LH-8 group (great improvement in 7.7%, moderate in 88.5%, and stable in 3.8%). The results were apparent in 3 months, more significantly results after 6 months compared to the control group. Authors conclude that LH-8 appears to be an effective and safe alternative to minoxidil topical treatment for AGA in females.

			No significant adverse events were reported and the quality of life, assessed with DLQI, improved in patients of the LH-8 group, while it worsened in the control group.

The applicant did not present a dose-finding study. No secondary pharmacology studies were submitted. No data on pharmacodynamic interactions with other medicinal products or substances were provided.

2.6.3. Discussion on clinical pharmacology

Cinainu is a combination product that contains preparations of four herbal substances including *Allium cepa* (onion), *Citrus limon* (lemon), *Theobroma cacao* (cacao) and *Paullinia cupana* (guarana). The applicant's rationale for the necessity of all active substances in the product is based on literature data only. The applicant has not provided data in support of the necessity of all extracts in the product in the proposed concentrations. There is no dedicated study to support the claimed mechanism of action, and the applicant refers to anti-inflammatory and immune modulating effects based on *in vivo* and *in vitro* data in literature. The exact mechanism of action for the combination is unknown. No dedicated pharmacodynamic studies were presented. Importantly, it is not known if the composition of LH-8 and CG 210 in these studies corresponds to the composition of Cinainu in this MAA. With respect to the pharmacokinetic profiles of the constituents of the product, these have not been fully characterised.

2.6.4. Conclusions on clinical pharmacology

In conclusion, the proof-of-concept and the pharmacokinetic profile of Cinainu in the treatment of AA in children and adolescents has not been sufficiently presented by the applicant.

2.6.5. Clinical efficacy

2.6.5.1. Dose response study

No dose finding study was performed in the proposed indication. The applicant states that posology is based on the size of the head only.

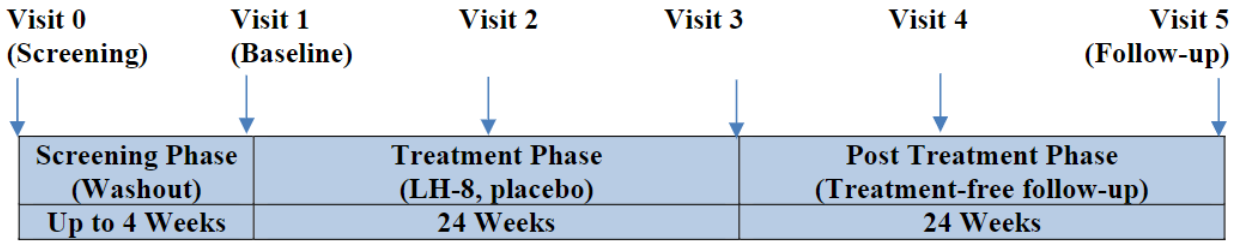
2.6.5.2. Main study

Title of study

Phase 2/3 clinical study for the treatment of moderate to severe scalp alopecia areata (AA) in children and adolescents (from 2 years to less than 18 years of age).

The single pivotal phase 2/3 RAAINBOW study is a randomised (2:1), double-blind, vehicle-controlled multicentre 24 weeks study in parallel groups with a 24 weeks off-treatment follow-up period. At screening (Visit 0), subjects had to discontinue their previous treatment for AA, if any. Screening period was up to 28 days. The 24-week treatment phase was including assessment Visits 1 to 3, which were taken place at 12-

week intervals. At assessment Visit 1, eligible subjects were randomly assigned in a 2:1 ratio to receive LH-8 cutaneous solution or vehicle (placebo) twice daily for a 24-week treatment period. During the treatment phase the subjects had drug diaries to be completed daily. The post-treatment safety and efficacy follow-up phase included Visit 4 and Visit 5, 12 and 24 weeks after end of treatment, respectively. Subjects and parents were instructed to contact the investigator, if an event on scalp occurred during the treatment or post-treatment period. They were asked to come to the site for an unscheduled visit, in order to perform additional examinations.



The RAAINBOW study protocol is based on the Uniform Core Protocol (UCP) for AA, which was developed in 2015 by the National Alopecia Areata Foundation (NAAF) to "simplify and promote future clinical trials for alopecia areata treatments". The applicant stated that RAAINBOW is the first phase 2/3 study to enrol not only children, but also moderate AA patients and to exclude patients with Alopecia Totalis, while all previous studies enrolled only patients with severe AA and Alopecia Totalis.

Methods

- **Study participants**

Inclusion criteria

1. Male and female children and adolescents aged 2 to less than 18 years.
2. Active scalp alopecia areata, involved 25% to 95% of the scalp (as measured by SALT score at screening).
3. Duration of hair loss between 6 months and 3 years.
4. Female subjects of childbearing potential (post-menarcheal) must had a negative urine pregnancy test at screening. Females of childbearing potential must either not be sexually active or be using an adequate birth control method throughout the duration of the study.
5. All subjects taking thyroid medication or hormonal therapy must be on a stable dose for 6 months and maintain such throughout the study.
6. Subjects must be willing to maintain the same hair style, including hair dye, throughout the study period.
7. Written informed consent signed.

Exclusion criteria

1. Hypersensitivity or intolerance to any active substances or excipients.
2. Any cause of hair loss other than AA.
3. Active scalp inflammation except AA.
4. Nevi, cutaneous or non-cutaneous lesions currently undiagnosed but suspicious for malignancy.
5. Female adolescents who are pregnant or who are nursing or plan pregnancy during the trial period.
6. Use of topical medication within 2 weeks prior to Visit 1.
7. Use of systemic AA therapies (e.g. prednisone, cyclosporine, methotrexate), including use of these medications for other indications, and intralesional corticosteroids within 1 month prior to Visit 1.
8. Administration of hydroxychloroquine or finasteride within two months prior to Visit 1.

9. Use of phototherapy, laser therapy or excimer laser on the scalp within three months prior to Visit 1.
10. Use of infliximab within 2 months, adalimumab within 3 months, and ustekinumab within 4 months prior to Visit 1 or use of other TNF inhibitors and biologic agents within 1 month or 5 half-lives before Visit 1.
11. Prior treatment with the product.
12. Evidence or history of alcohol, medication or drug abuse.
13. History of systemic or cutaneous medical, or psychiatric disease.
14. Participation in any other clinical trial within 30 days prior to Visit 1.
15. Subject is in a dependent relationship with the investigator's or sponsor's staff.
16. Any other condition or circumstance that, in the opinion of the investigator, could compromise the subject's ability to comply with the study protocol.

- **Treatments**

LH-8 cutaneous solution (0.126 ml per spray) applied to the whole scalp: Subjects aged 2 to ≤15 years: 7 sprays twice daily. Subjects older than 15 years: 8 sprays twice daily.

Placebo: vehicle (ethanol 96%, and purified water) applied to the whole scalp: Subjects aged 2 to ≤15 years: 7 sprays twice daily. Subjects older than 15 years: 8 sprays twice daily.

Duration of treatment: 24 weeks.

Objectives

Primary: To assess the therapeutic efficacy of a 24-week regimen of LH-8 cutaneous solution twice daily, in children and adolescents (2 to less than 18 years) with chronic moderate-to-severe scalp AA.

Secondary: To assess the efficacy, safety and tolerability of LH-8 cutaneous solution, including the ocular irritant potential and non-irritant potential on skin, the quality of life, acceptability and tolerability through self-evaluation as well as parental evaluation. To evaluate duration of the treatment effect after 6 months of treatment-free period. To assess the treatment effect on hair follicles in non-alopecic areas, the rate of spontaneous remission in placebo treated patients whose AA has been active for 6-12 months compared with those whose AA has been active for greater than 12 months.

- **Outcomes/endpoints**

Primary endpoint: Relative change in scalp alopecia areata severity scores (SALT) from baseline value to be assessed after 24 weeks of treatment. (Visual assessment and global standardised scalp photographs for SALT valuation.)

The relative change in SALT score will be calculated using the formula:

$$\text{Relative change in SALT score (\%)} = \frac{\text{SALT score at baseline} - \text{SALT score at endpoint}}{\text{SALT score at baseline}} \times 100\%$$

Key secondary efficacy endpoints: Absolute change in SALT score from baseline at the end of 24 weeks' treatment period, proportion of the responders, i.e. subjects achieving at least a 40% relative reduction in SALT score from baseline at the end of 24 weeks' treatment period.

Other secondary endpoints: Adverse events; general physical examination findings, including irritation of eyes and skin; evaluation of duration of treatment effect in responders, measured as relative SALT score changes from Visit 3, after 12 weeks and 24 weeks of treatment-free period; assessment of treatment effect

on hair follicles in non-alopecic areas by quantifying the number of new alopecic areas; assessment of the rate of spontaneous hair regrowth in placebo treated subjects with alopecia areata active for 6-12 months compared to those with alopecia areata active for more than 12 months; absolute and relative change from baseline in Children's Dermatology Life Quality Index (CDLQI) scores; change in percentage of subjects from baseline by the severity banding CDLQI scores; percentages of subjects by EuroQol Five Dimensions Youth Questionnaire (EQ-5D-Y) dimensions and levels at Visits 1-5; absolute and relative change of the EQ-Visual Analogue Scale (EQ-VAS) scores from baseline; evaluation of the paediatric AA Patient Benefit Index score.

- **Sample size**

The sample size calculation is based on the primary efficacy endpoint. In total, 87 subjects (58 in active and 29 in placebo group) would give 90.3% power, assuming a 24% difference in relative change in SALT score between the active and placebo groups, a probability of type I error of 0.05 (two-sided), common standard deviation of 32%, and randomisation ratio 2:1. To ensure adequate power for the primary analysis based on FAS, taking into account sample size calculation described above and assuming a 15% screening failure rate, as well as a 14% drop-out rate after randomisation, 120 subjects were to be screened in order to randomise 102 subjects and to have 87 subjects in FAS. As the number of drop-out was higher than expected a total of 107 subjects were randomised.

- **Randomisation and blinding**

At Visit 1, after confirming eligibility, subjects were randomly assigned to receive either LH-8 or placebo in a ratio 2:1. The randomisation numbers were blocked; the trial was double-blind. LH-8 and placebo solutions were identical in optical appearance, the packaging and labelling did not allow for any distinction. Placebo consisted of an identical product to LH-8, minus the herbal active ingredients. As extracts of herbal ingredients may have an odour, a specific process of extraction has been developed to neutralise it as much as possible. However, the sense of smell is a subjective matter; differences in perception may arise.

- **Statistical methods**

Analysis Populations

Intent to Treat Analysis (ITT) set is defined as all randomised subjects. Safety set (SS) is defined as all randomised subjects with at least one administration of IMP or placebo. Full Analysis Set (FAS) population includes all randomized subjects with at least one administration of IMP or placebo and fulfilling the main inclusion criterion on SALT score severity at baseline, comprised between SALT 25 and 95. The FAS was used as the primary population for all the efficacy analyses, and the ITT population was used as supportive.

Primary Analysis

The treatment groups were compared for the SALT score relative change from baseline at the end of 24-week treatment period using a Mixed Model for Repeated Measures (MMRM) analysis, including treatment, severity of alopecia at baseline (SALT 25-50, or SALT 50-95) as covariate based on the FAS analysis set.

The difference between treatment groups was estimated from least squares (LS) means. The accompanying p-value and two-sided 95% CI were provided. Patients with only one available evaluation at baseline were analysed using a method of multiple imputation (see section 'Handling of missing data' below).

Separate analysis using ANCOVA including treatment as a factor, severity of alopecia at baseline (SALT 25-50, or SALT 50-95) as covariates, and LOCF as missing data imputation method, was conducted to assess the robustness of the MMRM analysis results.

Other covariables such as duration of alopecia at baseline, and/or age, could be further analyzed to explore their influence on the primary outcome by adding the respective covariate to the MMRM and ANCOVA models.

If the basic assumptions of MMRM are not met, data was planned to be rank transformed, and a generalized linear model used as a main model. The model would have the rank of relative change as a dependent variable, treatment, visit, treatment by visit interaction as fixed effects and baseline severity as a covariate.

Handling of missing data

Considering that a non-negligeable number of patients withdrew from the study without any SALT score under treatment, it was decided (as described in the SAP version 2) to use a multiple imputation method for these patients before the use of the MMRM model. A pattern-mixture model was applied using the baseline SALT score, treatment (fixed effect), severity (random effect), and time visit (fixed effect). According to the protocol, the primary and secondary efficacy analyses were initially planned to be performed using a MMRM analysis under the assumption that data is missing at random. In order to evaluate robustness of the MMRM analysis results, further analysis of covariance (ANCOVA) was planned to be conducted using LOCF for missing data. Baseline value was to be imputed for subjects with no post baseline assessment of SALT score assuming that there was no change from baseline.

According to the protocol, subjects who discontinued the trial due to lack of efficacy, adverse events or with no post baseline SALT score were to be treated as non-responders in the responder analysis. For all other cases LOCF imputation method was to be applied in the responder analysis.

Secondary analyses

Key secondary endpoints included in the hierarchical testing:

- absolute change in SALT score from baseline at the end of 24 weeks treatment period
- proportion of subjects (responder rate) achieving at least a 40% relative reduction in SALT score from baseline at the end of 24 weeks treatment period.

Absolute change in SALT score was analysed using the same MMRM model as the primary endpoint. Responder rate in both groups was compared using Chi-Square test. The difference in proportions, stratified by SALT score (25-50%, 50-95%) and duration of alopecia (6-12 months and more than 12 months) at baseline, was estimated by the common risk difference together with its two-sided 95% CI. Additionally, for sensitivity purposes, the same analysis was provided with the following thresholds for the responder status: 30% and 50% relative reduction in SALT score from baseline at the end of 24 weeks.

Multiplicity

Only if the null hypothesis of the primary endpoint is rejected, the confirmatory testing of the hypotheses for the key secondary endpoints will be performed, in a hierarchical manner. In case superiority based on the primary efficacy endpoint is not shown the analysis of the secondary efficacy endpoints will be considered as exploratory only.

A hierarchical testing strategy for analysis of key secondary endpoints will be employed to preserve the type I error rate, i.e., the confirmatory testing of the hypothesis will stop when the statistically significant result is not achieved, and hypothesis testing will be continued in an exploratory way.

Subgroup analysis

The primary endpoint (relative change from baseline of SALT score at 24 weeks) is planned to be analysed and presented with forest-plots in the following subgroups:

- Percentage of scalp affected at baseline e.g. but not limited to 25-50%/>=50%
- AA duration at baseline: 6 - 12 months and more than 12 months
- Age e.g. but not limited to: <12yo/>12yo
- Skin comorbidities
- AA activity prior the enrolment: subject stable at V0 or those who were in active phase at V0
- Gender
- Number of relapses

Interim analysis

No interim analysis was planned.

SAP governance

The first SAP version was authored in April 2022 and then updated twice during October 2022. According to the CSR synopsis, the last patient enrolled was in September 2018. The trial was in total 52 weeks long for an individual subject.

Main changes from the protocol specified analyses

According to the SAP, few changes were made:

- The FAS has been introduced for the main efficacy analysis in place of the ITT population.
- In order to handle missing data under a missing not at random assumption which is clinically reasonable, a multiple imputation method was introduced to be used for the main analysis of the primary and the two key secondary endpoints.
- The per-protocol analysis set which was predefined in the protocol was deemed not reliable as the number of patients strictly corresponding to the target population according to the SAP baseline severity was quite reduced and lead to a restricted FAS population.

In summary, the following was reported which concerns the analysis methods:

Before performing planned MMRM analysis, the normality assumption of the data was checked, which is an assumption of MMRM, by using normal Q-Q plot and Shapiro-Wilk test. Based on these two tests results, it was confirmed that the primary and the first secondary endpoint in FAS population did not follow normal distribution. Therefore, a generalised linear model was given a preference over the MMRM. The model was run based on GLIMMIX procedure in SAS. For this purpose, data was rank transformed by visit and then generalized linear model was used.

For the primary endpoint, the GLIMMIX model had the rank of relative change from baseline in SALT score as the dependent variable and treatment, visit, treatment by visit interaction as fixed effects, and baseline severity based on SALT score as a covariate. Similar corresponding GLIMMIX model was used for the absolute change from baseline in SALT score.

MMRM approach is provided for information since it is not planned in case of non-normality. To confirm that the product delivers efficacy, an ANCOVA analysis is also performed.

Results

- **Participant flow**

A total of 120 patients were screened in the RAAINBOW trial; the below Table 4 summarises subject disposition.

Table 4. Summary of subject disposition in RAAINBOW study

Population	LH-8	Placebo	Overall
Intent to treat (ITT) Population	71	36	107
Full Analysis Set (FAS) Population	42 (59.2%)	20 (55.6%)	62 (57.9%)
Completed Population	51 (71.8%)	25 (69.4%)	76 (71.0%)
Safety Population	71 (100%)	36 (100%)	107 (100%)
Per-Protocol (PP) Population	46 (64.8%)	25 (96.2%)	71 (66.4%)

Note: Percentages are based on the number of subjects in ITT population in each treatment arm.

The intent to treat (ITT) analysis set is defined as all randomised subjects. The Full Analysis Set (FAS) population includes all randomised subjects with at least one administration of treatment or placebo and fulfilling the main inclusion criterion on SALT score severity at baseline, comprised between SALT 25 and 95. The completers set (CS) is defined as all subjects included in the SS who completed study as scheduled. The per protocol set (PPS) is defined as all subjects included in the SS who have no major protocol deviations.

Of the 62 subjects of the FAS population, 10 dropped out before the visit 3 at 24 weeks, of which 9 belonging to LH-8 group and 1 to placebo group leading to 52 subjects completing the 24-weeks' treatment period, 33 in the LH-group and 19 in the placebo group. These subjects dropped-out during or around Covid-lockdows, or major Covid waves.

- **Recruitment**

First patient was enrolled on 01 Feb 2018. Last patient enrolled on 07 Sept 2018. Last Patient Last Visit (LPLV) was on 14 Sept 2022. The last patient completed the study in Sept, 2022.

- **Conduct of the study**

There have been several protocol amendments. In order to keep as much subjects as possible in the study during Covid-lockdowns, or major Covid waves, an amendment to allow home-pictures was put in place. Five major protocol deviations have been identified by the Applicant in the Table 5 below.

Table 5. Major protocol deviations

No.	Random No.	V1	V2	V3	V4	V5	Description of Protocol deviation	PD Grading
1.		X					The photos for SALT assessment were taken during V1, however a green screen instead of a blue one was used. Further, the photos were not uploaded until MV01. V1 photos don't show all alopecic areas at once for each side as hair clips were not used when taking the photos.	major
2.				X			At patient's V3 the site has missed to perform photography of the occipital area and no SALT assessment was performed.	major
3.				X			SALT score was scored at 0% although the subject is completely bald. SALT score should be 100%. Mistake/inversion made by the PI	major
4.				X			volunteer shaved the entire occipital parietal part (10 days before visit 3), so in the photos we can see a difference between V2 and V3. However there was an improvement compared to V2. The PI took into account the shaving and based his evaluation on the severity and not the photo which are not representative	major
5.						X	The same SALT score for V4 and V5 (74,6%) although the clinical features are different	major

- **Baseline data**

The Full Analysis Set (FAS) population is presented below.

Table 6. Baseline characteristics of FAS population

Characteristics	LH-8 (N=42)	Placebo (N=20)
Age at screening (Years)		
Mean	11.14	10.10
Min; Max	(4.00, 17.00)	(3.00, 17.00)
SD	3.84	4.02
Median	11.50	9.50
Female	22 (52.4)	12 (60.0)
Male	20 (47.6)	8 (40.0)
American Indian or Alaska Native	0	1 (5.0)
Asian	12 (28.6)	6 (30.0)
Black or African American	5 (11.9)	1 (5.0)
Native Hawaiian or Other Pacific Islander	1 (2.4)	0
Unknown	0	1 (5.0)
White	24 (57.1)	11 (55.0)
Time since onset of alopecia areata - Mean yrs		
Mean	3.3	2.5
Min; Max	(0.5, 10.5)	(0.5, 11.1)
SD	2.83	2.57
Gender n(%)		
Number of flares (including current)	2.0	2.0
SD	1.44	1.36
Severe AA at baseline (SALT 50-95) n° (%)	24 (57%)	13 (65%)
Moderate AA at baseline (SALT 25-50) n° (%)	18 (43%)	7 (35%)
SALT score – All subjects		
Mean	56.1	61.8
Min; Max	(25.2, 93.3)	(27.9, 92.3)
SD	21.1	21.4
SALT score - Severe AA subjects at baseline		
Mean	71.2	74.8
Min; Max	(50.4, 93.3)	(50.1, 92.3)
SD	14.4	12.9
SALT score - Moderate AA subjects at baseline		
Mean	36.0	37.6
Min; Max	(25.2, 48.9)	(27.9, 47.4)
SD	6.6	8.7

Concomitant products used in the FAS population is presented below.

Table 7. Summary of concomitant medications in the FAS population.

Medications	LH-8(N=42) n(%)	E	Placebo(N=20) n(%)	E
Advantan creme/methylprednisolone 0.1%	0		1 (5.0%)	1
Advantan ointment/Methylprednisolone	0		1 (5.0%)	1
Aerius	0		1 (5.0%)	1
Alex Syrup	0		1 (5.0%)	1
Antigone 75	0		1 (5.0%)	1
Azithromycin	1 (2.4%)	1	0	
Bronchipret juice	1 (2.4%)	1	0	
Cetirizine	0		1 (5.0%)	1
Cleanance Comedomed	0		1 (5.0%)	1
Clobex shampoo	1 (2.4%)	1	0	
Comirnaty/ Biontech vaccine	1 (2.4%)	1	0	
Defluval	1 (2.4%)	1	0	
Dermoval gel	0		1 (5.0%)	2
Dimenhydrinate	1 (2.4%)	1	0	
Diprosone cream	1 (2.4%)	1	0	
Drotin M	0		1 (5.0%)	1
Efferalgan	0		1 (5.0%)	1
Emeset	0		1 (5.0%)	1
Erythro gel	1 (2.4%)	1	0	
Ethlnylestradiol	1 (2.4%)	1	0	
Granudoxy	0		1 (5.0%)	1
Honitus	1 (2.4%)	1	0	
IBUPROFEN	1 (2.4%)	1	0	
Ibuprofen	1 (2.4%)	2	0	
Ibuprofen/Ibulysin ratiopharm	0		1 (5.0%)	1
Ibuprofene 400	1 (2.4%)	1	0	
Immodium akut/ Loperamide	1 (2.4%)	1	0	
Levothyrox	1 (2.4%)	1	0	
Locoid lotion	1 (2.4%)	1	0	
Mometasone	1 (2.4%)	1	0	

Medications	LH-8(N=42) n(%)	E	Placebo(N=20) n(%)	E
NOVAPHANE	1 (2.4%)	1	0	
Norflox Oz	1 (2.4%)	1	0	
Nose drop	0		1 (5.0%)	1
Nurofen juice(Ibuprofen)40mg/ml	1 (2.4%)	2	0	
Nurofen liquid/ Ibuprofen	0		1 (5.0%)	1
Nurofen syrup/Ibuprofen 4%	1 (2.4%)	1	0	
ORS SACHET	1 (2.4%)	1	0	
Oflox Oz	0		1 (5.0%)	1
PRORHINEL	1 (2.4%)	1	0	
Paracetamol	4 (9.5%)	6	1 (5.0%)	2
Ranidom	0		1 (5.0%)	1
Sensinol Shampoo	0		1 (5.0%)	1
Soderm/Betamethasone	0		1 (5.0%)	1
Soframycin lotion	1 (2.4%)	1	0	
Sporlac	0		1 (5.0%)	1
VOMISTOP	1 (2.4%)	1	0	
Ventolin syrup	1 (2.4%)	1	0	
Ventoline	1 (2.4%)	1	1 (5.0%)	1
Vigantoletten Vitamin D	1 (2.4%)	1	1 (5.0%)	1
Vitamin C	1 (2.4%)	1	0	
Vitamin D	2 (4.8%)	2	0	
Vomex/Dimenhydrinat	1 (2.4%)	1	0	
XYZAL	1 (2.4%)	1	0	
Zinc	1 (2.4%)	1	0	
mucosolvan	0		1 (5.0%)	1
nurofen fiebersoft	0		1 (5.0%)	1

[1] Percentage will be computed using N provided in the Column header, n is the number of subjects. E is the number of medications.

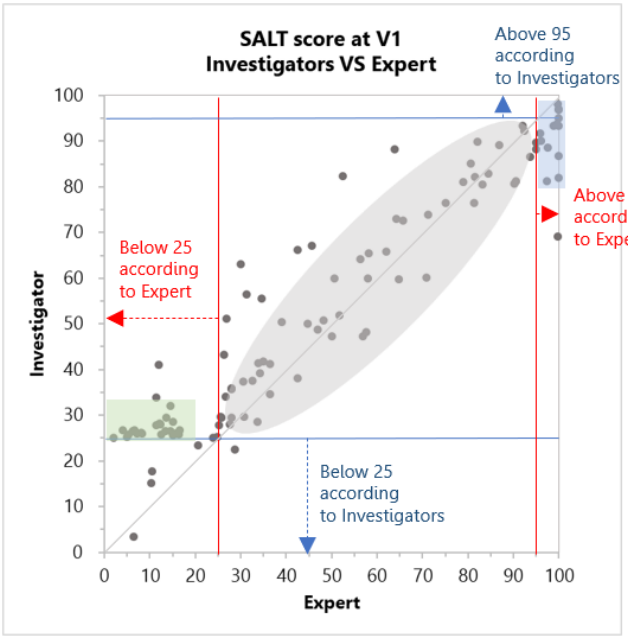
- **Numbers analysed**

Randomised (ITT): 107 2:1 (LH-8: 71; Placebo:36). Analysed (FAS): 62 (LH-8: 42; Placebo:20).

After the last-patient-last-visit (Sept 2022), while the queries resolution was ongoing on blinded data, and before the Blind review (Oct 14, 2022), two issues related to patient's inclusion were identified: both, patients with mild AA, and with Alopecia Totalis were incorrectly included in the trial. These subjects were therefore excluded from the FAS population, which explains the difference (ITT (n=107) and FAS (n=62)). Two reasons, identified before unblinding, explain these incorrect inclusions:

- Screening and decision for inclusion was based on SALT score at V0 and treatment was provided at V1, which took place up to a month after V0. During this period, the patient's alopecia evolved, and in certain cases, the patient's SALT score became out of the inclusion criteria. To avoid experimental bias, the SALT score was considered for inclusion purpose at screening only if the latter was confirmed at V1 as being still within the admitted inclusion criteria of SALT. Five patients rated just above SALT 25 at V0 were rated below SALT 25 at V1 - by the same investigator. Also, three patients rated just below SALT 95 at V0 were rated above SALT 95 at V1 (i.e Alopecia Totalis).
- 26 patients, the investigators rated SALT of V1 visit just above the inclusion threshold of SALT 25 (blue line), while the independent expert rated the same patient/visit below or far below SALT 25 (red line). This discrepancy can be observed in the green area of the below Figure 1.

Figure 1. SALT score discrepancy between Investigator and Expert at Visit 1 (from 2.5-4)



These 26 subjects were hence excluded from the FAS population, while being included in the ITT population.

Discrepancies were also identified in 11 subjects rated by the investigator just below the inclusion threshold of SALT 95, which the expert rated above SALT 95 (*i.e* Alopecia Totalis), and confirmed by picture checks. Overall, the SALT score at V1 from the independent expert was considered only for inclusion decision in the FAS population. For the statistical analysis of all patient populations (ITT, FAS, PP, Safety), the SALT score used at V1 (baseline), V2, V3 (primary end-point), V4 and V5 (follow-up) are the ones from the investigators. The Blind review meeting during which the decision to include in the FAS population only patients rated as SALT 25-95 by the independent expert took place once the recruitment was over. Although the study was powered (90% power) with 87 evaluable patients, it is now based on 62 (FAS).

After unblinding, 17 patients belonged to the LH-8 group and 9 to the placebo group. The applicant argues that this is consistent with the overall population split between drug and placebo (2:1). 100% of the 9 patients under placebo did improve. The applicant states that this confirms that when AA is still at mild stage, the chance for spontaneous recovery is high, as mentioned in the literature.

- **Outcomes and estimation**

The results provided in the initial MAA are now considered invalid and incorrect by the applicant, see below.

Table 8. Overview of the results of the efficacy study i.e., the RAAINBOW study in AA in children and adolescents in the FAS population

Study	Treatment Arm	FAS # Enrolled / Completed	Primary Endpoint Relative change in SALT score from baseline value to be assessed after 24 weeks of treatment	Secondary Endpoints Absolute change in SALT score from baseline value to be assessed after 24 weeks of treatment	Secondary Endpoints Proportion of "responders" (subjects achieving at least a 40% relative reduction in SALT score after 24 weeks)
RAAINBOW	LH-8	42 / 33	+22.87%	+12.72	26.2%
	Placebo	20 / 19	-8.00% P<0.0001	-0.77 P=0.0013	5% P=0.0484

The applicant engaged an external consultancy to conduct an audit of the primary analysis, which uncovered critical deviations from the analyses prespecified in the SAP and from the established statistical principles for longitudinal studies. Consequently, the efficacy results submitted in the initial MAA and in the response to the CHMP D120 LoQ were considered invalid and incorrect by the applicant, and as stated during the OE, the new results from the re-analysis presented as part of the applicant's response to the CHMP Day 180 LoOI are no longer considered preliminary (presented in the Table 9 below).

Table 9. Results from the re-analysis presented as part of the applicant's response to the CHMP Day 180 LoOI

Study	Treatment Arm	FAS # Enrolled / Completed	Primary Endpoint Relative change in SALT score from baseline value to be assessed after 24 weeks of Treatment FAS population	Secondary Endpoints Absolute change in SALT score from baseline value to be assessed after 24 weeks of treatment	Secondary Endpoints Proportion of "responders" (subjects achieving at least a 40% relative SALT score after 24 weeks)
RAAINBOW	LH-8	42 / 33	+15.47%	+9.33	26.19%
	Placebo	20 / 19	-10.83% P=0.049	-2.23 P=0.081	5% P=0.0488

The summary of main efficacy results was hence updated accordingly, and the re-analysed data are discussed under 'Discussion on clinical efficacy' and 'Benefit risk assessment'.

- **Ancillary analyses**

In general, the subgroups are too small to draw reliable conclusions.

- **Summary of main efficacy results**

The following Table 10 summarise the efficacy results from the main study supporting the application. These summaries should be read in conjunction with the discussion on clinical efficacy and benefit risk assessment.

Table 10. Summary of efficacy for the single pivotal RAAINBOW study as provided in the initial MAA submission (considered invalid and incorrect by the applicant as stated in the Oral Explanation)

Title: Double-blind, vehicle-controlled, randomised, multi-centre study to evaluate the efficacy and safety of LH-8 cutaneous solution in children and adolescents with moderate to severe scalp alopecia areata			
Study identifier	EudraCT No: 2016-003208-30		
Design	Double-blind, vehicle-controlled, randomised, multi-centre study 24 weeks treatment followed by 24-weeks treatment-free		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		48 weeks 2 to 28 days not applicable
Hypothesis	To show superiority of LH-8, H0: u(r)LH-8 – u(r)Plac=0%; Ha: u(r)LH-8 – u(r)Plac≠0%; where μ(r) denotes the mean relative change in SALT score from baseline at the end of 24 weeks' treatment period.		
Treatments groups	LH-8 cutaneous solution Cinainu		24 weeks treatment + 24 weeks treatment-free follow-up FAS N=42 ITT N=71
	Placebo		24 weeks treatment + 24 weeks treatment-free follow-up FAS N=20 ITT N=36
Endpoints and definitions	Primary endpoint	RChange	To assess the therapeutic efficacy of a 24-week regimen of administration of LH-8 cutaneous solution twice daily, in children and adolescents (2 to less than 18 years) with chronic moderate-to-severe scalp alopecia areata
	Key secondary	AChange	Absolute change in SALT score from baseline at the end of 24 weeks' treatment period
	Key secondary	Resp	Proportion of the responders, i.e. subjects achieving at least a 40% relative reduction in SALT score from baseline at the end of 24 weeks' treatment period
	Other secondary	AEs	Adverse events
	Other secondary	GenPhy	General physical examination findings, including irritation of eyes and skin

	Other secondary	TmtEffect	Evaluation of duration of treatment effect in responders, measured as relative SALT score changes from Visit 3 (end of treatment) after 12 weeks (Visit 4) and 24 weeks (Visit 5) of treatment-free period. (Visual assessment and global standardised scalp photographs for SALT evaluation.)
	Other secondary	NewArea	Assessment of treatment effect on hair follicles in non-alopecic areas by quantifying the number of new alopecic areas
	Other secondary	SponReg	Assessment of the rate of spontaneous hair regrowth in placebo treated subjects with alopecia areata active for 6-12 months compared to those with alopecia areata active for more than 12 months. (Visual assessment and global standardised scalp photographs for SALT evaluation)
	Other secondary	CDLQI	Absolute and relative change from baseline in Children's Dermatology Life Quality Index (CDLQI) scores
	Other secondary	EQ5D	Percentages of subjects by EuroQol Five Dimensions Youth Questionnaire (EQ-5D-Y) dimensions and levels at Visits 1-5.
	Other secondary	EQVAS	Absolute and relative change of the EQ-Visual Analogue Scale (EQ-VAS) scores from baseline.
	Other secondary	PAABI	Evaluation of the Paediatric Alopecia Areata Patient Benefit Index (PAAPBI) scores at Visits 1-5.
Database lock	8 Nov 2022		
Results and Analysis			
Analysis description	**Primary Analysis**		

<table>
<tr><td>Analysis population and time point description</td><td colspan="3">ITT = 107

Full Analysis Set (FAS) = 62

Difference of 45 subjects between ITT and FAS explains as follows.

Among inclusion criteria, subjects had to have alopecia areata (AA) involving 25% to 95% of the scalp (SALT 25-95), Moderate AA corresponding to SALT 25-50 and Severe AA corresponds to SALT 50-95.

The first treatment (V1) occurred between 2 and 28 days after screening (V0).

In 5 subjects, investigator assessed SALT above 25 at enrolment (V0) and below 25 at inclusion (V1).

In 3 subjects, investigator assessed SALT below 95 at enrolment (V0) and above 95 at inclusion (V1).

Before unblinding, all 7 subjects were excluded from the FAS population.

As planned in the protocol, an expert, Dr Bianca-Maria Piraccini, then President of the European Hair Research Society (https://ehrs.org/about/), evaluated all pictures to determine an independent SALT score, allowing to double-check the investigators' SALT score.

In 26 subjects, the independent expert assessed the SALT score at V1 below 25 (11.5 on average). Based on individual pictures assessment, it was decided during the Blind review meeting (before unblinding) not to include in the FAS population all patients rated SALT < 25 by the independent expert.

Similarly, in 11 subjects, the independent expert assessed V1 SALT score above 95 (98.6 on average). Based on individual pictures assessment, it was also decided during the Blind review meeting (before unblinding) not to include in the FAS population all patients rated SALT > 95 by the independent expert at inclusion (V1).

Considering that the effect size (relative change in SALT score between active and placebo groups) was superior to what was initially estimated (30.87% *vs* 24%), the current sample size of 62 subjects leads to an actual power of 78.83%.</td></tr>
<tr><td rowspan="7">Descriptive statistics and estimate variability</td><td>Treatment group</td><td>LH-8</td><td>Placebo</td></tr>
<tr><td>Number of subject</td><td>42</td><td>20</td></tr>
<tr><td>RChange
Mean</td><td>+22.87%</td><td>-8%</td></tr>
<tr><td>AChange
Mean</td><td>+12.72</td><td>-0.77</td></tr>
<tr><td>Resp
Percentage</td><td>26.2%</td><td>5%</td></tr>
<tr><td>TmtEffect
Mean</td><td>+15.27%</td><td>-26.87%</td></tr>
<tr><td>NewArea
Proportion</td><td>21.3%</td><td>68.5%</td></tr>
</table>

During the OE, the applicant stated that the new results from the re-analysis presented as part of the response to the Day 180 LoOI are no longer considered preliminary and are presented in the Table 11 below.

Table 11. Double-blind, vehicle-controlled, randomised, multi-centre study to evaluate the efficacy and safety of LH-8 cutaneous solution in children and adolescents with moderate to severe scalp alopecia areata

Title: Double-blind, vehicle-controlled, randomised, multi-centre study to evaluate the efficacy and safety of LH-8 cutaneous solution in children and adolescents with moderate to severe scalp alopecia areata			
Study identifier	EudraCT No: 2016-003208-30		
Design	Double-blind, vehicle-controlled, randomised, multi-centre study 24 weeks treatment followed by 24-weeks treatment-free		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		48 weeks 2 to 28 days not applicable
Hypothesis	To show superiority of LH-8, H0: u(r)LH-8 – u(r)Plac=0%; Ha: u(r)LH-8 – u(r)Plac≠0%; where μ(r) denotes the mean relative change in SALT score from baseline at the end of 24 weeks' treatment period.		
Treatments groups	LH-8 cutaneous solution Cinainu		24 weeks treatment + 24 weeks treatment-free follow-up FAS N=42 ITT N=71
	Placebo		24 weeks treatment + 24 weeks treatment-free follow-up FAS N=20 ITT N=36
Endpoints and definitions	Primary endpoint	RChange	To assess the therapeutic efficacy of a 24-week regimen of administration of LH-8 cutaneous solution twice daily, in children and adolescents (2 to less than 18 years) with chronic moderate-to-severe scalp alopecia areata
	Key secondary	AChange	Absolute change in SALT score from baseline at the end of 24 weeks' treatment period
	Key secondary	Resp	Proportion of the responders, i.e. subjects achieving at least a 40% relative reduction in SALT score from baseline at the end of 24 weeks' treatment period

	Other secondary	AEs	Adverse events
	Other secondary	GenPhy	General physical examination findings, including irritation of eyes and skin
	Other secondary	TmtEffect	Relative change in SALT score from baseline to Week 48
	Other secondary	NewArea	Assessment of treatment effect on hair follicles in non-alopecic areas by quantifying the number of new alopecic areas
	Other secondary	SponReg	Assessment of the rate of spontaneous hair regrowth in placebo treated subjects with alopecia areata active for 6-12 months compared to those with alopecia areata active for more than 12 months. (Visual assessment and global standardised scalp photographs for SALT evaluation)
	Other secondary	CDLQI	Absolute and relative change from baseline in Children's Dermatology Life Quality Index (CDLQI) scores
	Other secondary	EQ5D	Percentages of subjects by EuroQol Five Dimensions Youth Questionnaire (EQ-5D-Y) dimensions and levels at Visits 1-5.
	Other secondary	EQVAS	Absolute and relative change of the EQ-Visual Analogue Scale (EQ-VAS) scores from baseline.
	Other secondary	PAABI	Evaluation of the Paediatric Alopecia Areata Patient Benefit Index (PAAPBI) scores at Visits 1-5.
Database lock	8 Nov 2022		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Screened: 120 Assessed for eligibility: 117 Randomised (ITT): 107 Analysed (FAS): 62 (LH-8: 42; Placebo:20)		
Descriptive statistics and estimate variability	Treatment group	LH-8	Placebo
	Number of subject	42	20
	RChange Mean	+15.57%*	-10.83%
	AChange Mean	+9.33	-2.23

	Resp Percentage	26.19%	5%
	TmtEffect Mean	+40.89%	+1.51%
	NewArea Proportion	21.3%	68.5%
	SponReg Proportion	n.a	AA 6-12 months = 15% AA > 12 months = 35%
	CDLQI Change	-1.84	+1.12
	EQ5D	cf EQVAS	cf EQVAS
	EQVAS Change	+4.37	-9.50
	PAABI Change	2.00	1.06
Effect estimate per comparison	Primary endpoint RChange	Comparison groups	LH-8 *vs* Placebo
		Difference between group	26.31 percentage points
		P-value FAS	0.049
	Key secondary AChange	Comparison groups	LH-8 *vs* Placebo
		Difference between group	11.55
		P-value FAS	0.081
	Key secondary Resp	Comparison groups	LH-8 *vs* Placebo
		Difference between group	21.2 percentage points
		P-value Chi-square test	0.0488
	CDLQI	Comparison groups	LH-8 *vs* Placebo
		Difference between group	2.96
		P-value T-test for continuous variables	0.0441
	EQVAS	Comparison groups	LH-8 *vs* Placebo
		Difference between group	13.87
		P-value	0.0362

		T-test for continuous variables	
	PAABI	Comparison groups	LH-8 *vs* Placebo
		Difference between group	0.94
		P-value T-test for continuous variables	Non-significant
Note	In the ITT population, the primary analysis of the primary endpoint, the relative change in SALT score from baseline to Week 24, using the prespecified parameterization of the linear MMRM model showed a numerical difference in the expected direction, but this difference was not statistically significant: mean difference +17.31%, p=0.35. To explore potential moderators of the treatment effect, multivariate analyses were planned in the SAP. In this perspective, to evaluate whether the treatment effect in the primary endpoint was influenced by whether patients met the baseline SALT score inclusion criterion in the ITT population, a treatment-by-baseline-SALT-score-fulfilment interaction term was added to the MMRM model used for the primary analysis. It was observed that almost all p-values obtained for the interaction term were below 0.05, indicating a significant treatment-by-baseline-SALT-score-fulfilment interaction. When such an interaction is present, the treatment effect observed in the overall ITT population may not accurately reflect the true effect, as the statistical significance and magnitude of the treatment effect in the ITT can vary based on the nature and extent of the interaction, the overall sample size, and the proportion of patients who meet the baseline SALT score inclusion criterion within the ITT population.		
Analysis description	Sample size: The sample size calculation is based on the mean relative change in SALT score from baseline at the end of 24 weeks' treatment period. 87 subjects in total (i.e. 58 subjects for active and 29 subjects for placebo group) would give 90.3% power, assuming 24% difference in relative change in SALT score between the active and placebo groups, a probability of type I error of 0.05 (two-sided), common standard deviation of 32%, and randomisation ratio 2:1. To assure adequate power for the primary analysis based on the intent to treat (ITT) as well as completer set (CS), taking into account sample size calculation described above and assuming a 15% screening failure rate, as well as 14% of drop-out rate after randomisation, 120 subjects will be screened in order to randomise 102 subjects and to have 87 completers in total. Analysis sets: The safety set (SS) was defined as all randomised subjects with at least one administration of IMP.		

	The ITT analysis set was defined as all randomised subjects. The full analysis set (FAS) was the primary analysis population for efficacy and was defined as all randomised subjects who had a SALT score between 25 and 95 at the time of randomization, based on assessments by both the investigators and the experts. Efficacy: The primary analysis of the primary endpoint was conducted in the FAS and used a Mixed Model for Repeated Measures (MMRM), including treatment, visit, treatment-by-visit interaction, severity of alopecia at baseline (four categories: SALT<25, SALT 25-50, SALT 50-95, SALT>95) as covariate. The difference between treatment groups was estimated from least squares (LS) means. The accompanying p-value and two-sided 95% confidence interval (CI) were provided. An unstructured covariance matrix pattern was used to estimate the within-subject variation over repeated measures. Model parameters were estimated using restricted maximum likelihood (REML) method and Kenward–Roger degrees of freedom. The primary and secondary efficacy analyses were performed using a MMRM analysis under the assumption that data is missing at random. Considering that a non-negligeable number of patients withdrew from the study without any SALT score under treatment, it was decided to use a Multiple Imputation method for these patients, before the use of the MMRM model. A pattern-mixture model will be applied, using the baseline SALT score, treatment, severity, time visit. In addition, the primary endpoint analysis will be also performed on the ITT population. Separate analysis using analysis of covariance (ANCOVA) including treatment as a factor, severity of alopecia at baseline (SALT 25-50, or SALT 50-95) as covariates, and last observation carried forward (LOCF) as missing data imputation method, will be conducted to assess the robustness of the MMRM analysis results. Other covariables such as duration of alopecia at baseline, and/or age, might be further analysed to explore their influence on the primary outcome by adding the respective covariate to the MMRM and ANCOVA models. The SALT score values will also be listed at each visit for each subject. Only if the null hypothesis of the primary endpoint is rejected, the confirmatory testing of the hypotheses for the key secondary endpoints will be performed, in a hierarchical manner. In case superiority based on the primary efficacy endpoint is not shown the analysis of the secondary efficacy endpoints will be considered as exploratory only. To find predictive parameters of the response of the disease, multivariate analyses were planned. In this perspective, baseline-SALT-score-fulfilment

	interaction was performed. Sensitivity analyses were performed to assess the risk of bias due to missing data using different assumptions and imputation methods, including control-based pattern multiple imputation (missing not at random (MNAR) assumption), Baseline Observation Carried Forward (baseline-observation-carried-forward (BOCF) - MNAR assumption), multiple imputation (missing at random (MAR) assumption), and Observed Cases. A t-test was used for the absolute and relative change from baseline in CDLQI scores and absolute and relative change of the EQ-Visual Analogue Scale (EQ-VAS) scores from baseline. The proportion of patients who reached SALT ≤ 20 and SALT ≤ 10 was evaluated.

** Correct value considered by CHMP: +15.47%*

2.6.5.3. Clinical studies in special populations

The pivotal phase 2/3 RAAINBOW study included children and adolescents from 2 years of age to 17 years of age. There were no studies in other special populations.

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable.

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

Not applicable.

2.6.5.6. Supportive study

Not applicable.

2.6.6. Discussion on clinical efficacy

The clinical programme relies mainly on one single pivotal, phase 2/3 RAAINBOW study, which is a randomised, double-blind, vehicle-controlled multicentre 24 weeks study in parallel groups with a 24 weeks off-treatment follow-up period. The applicant utilises Full Analysis Set (FAS) population of this study n=62, although a minimum of 87 subjects were planned to be included in the FAS (see below). Importantly, RAAINBOW was carried out without the active substances being adequately characterised, and the manufacturing of the active substances was changed for the now proposed product, subject to this MAA. The herbal preparations in the conducted trial were not manufactured under GMP conditions. The applicant provided analysis to attempt to demonstrate that the trial batches are the same as those for the intended product. All changes between the batches in the RAAINBOW study, the stability batches and the intended product were described and their potential impact on the intended product efficacy and safety analysed. The issue is thoroughly discussed under Quality section. Overall, the applicant has not demonstrated that the

medicinal product applied for corresponds to what was tested in the RAAINBOW study. Consequently, statements about the clinical efficacy of Cinainu cannot be derived from the RAAINBOW study.

Furthermore, according to the EMA guideline on single pivotal trials (CPMP/EWP/2330/99), to be acceptable, the study will have to be exceptionally compelling. Hence, efficacy of Cinainu in the AA indication in children and adolescents is insufficiently substantiated and has not been established with the level of evidence normally expected for a single pivotal study in a MAA. This is further discussed below.

Design and conduct of clinical studies

There have been several protocol amendments for the RAAINBOW study. The applicant submitted a summary and rationale of all changes from the first protocol used to the latest version. The primary study objectives of the RAAINBOW study i.e. relative change in SALT scores from baseline value, is considered adequate, as are the inclusion and exclusion criteria (male and female children and adolescents aged 2 to less than 18 years with active AA involving 25% to 95% of the scalp between 6 months and 3 years in duration were included in the study). In the demographics of the patient population, the mean of 'Time since AA onset (years)' is above, or close to three years, but the selected study population is considered representative of the target population, as reflected in the proposed indication. However, in milder forms of AA the chance of spontaneous recovery is increased in contrast to extensive AA. The chance of spontaneous recovery is more likely with a short disease duration. Patients with other autoimmune diseases (incl. autoimmune vitiligo, lupus erythematosus, psoriasis and rheumatoid arthritis) could be included in the RAAINBOW study as these diseases were not mentioned in the exclusion criteria. According to the applicant, none of the included patients had these before or during the study. However, it is noted that one patient in the placebo group was diagnosed with Hashimoto's disease and one patient in the LH-8 group had hypothyroidism, diseases of which thinning hair is a common symptom. Hence, the applicant's explanation is not supported.

The scientific rationale for the posology in children and adolescents was not convincingly shown. The applicant states that the difference in applied quantity is based on the size of the head scalp, and hence is based on the volume applied, and not the amount of active substance. Furthermore, the posology in children and adolescents 2-<18 years of age in AA was mainly based on considerations from non-clinical *in vitro* data and evaluation on patients with AGA. No dedicated dose-finding studies are presented to support the posology in the AA in children and adolescents (2-<18y). Subjects and their parents were instructed to apply the cutaneous solution by spraying on the whole scalp applicator and to gently massage scalp after application. The participants were allowed to wash the hair 30 minutes after administration. The applicant's justification for treating the whole scalp and not only areas without hair is based on the proposed mode of action and the benign safety profile of the product. As claimed, the study treatments were identical in optical appearance and a specific process of extraction to neutralise the odour of the active ingredients was used. However, there was an obvious risk of unblinding due to the odour of the active ingredients in the LH-8 treatment, which could have a major impact on the study results. According to the applicant, the manufacturing process includes steps to capture the volatile and reactive sulphur compounds of the onion. The product contains 1% of lemon, which, as claimed, is the least odorant part and the dry powder of cocoa and guarana are odour-less. However, a proper test to substantiate this evaluation of the risk of unblinding due to the odour of all the active ingredients in the LH-8 treatment is missing.

The SALT score is usually used as a tool to assess the extent of patients' scalp hair loss, to measure the severity of AA, and the primary outcome of pivotal trial. However, the reliability and validity of centrally and investigator assessed SALT scores in the RAAINBOW study was questioned. The use of central independent readings to determine FAS does not only limit the analysis population which remains unacceptable but also

indicates a large discrepancy between investigator and central read which points to a high inter-reader variability of the SALT scores in this study. It was also questioned if the pictures taken by parents could have added to bias. The applicant stated that they assured that taking pictures at home did not have any impact on the safety or physical or mental integrity, the scientific value of the trial, the safety or rights of the subjects, or the primary endpoint robustness. Moreover, the clinical study protocol envisaged that the board of certified dermatologists-trichologists should determine SALT score independently from the investigator's assessment at each visit of the study. A reconciliation should have been performed between the assessments by different readers. This board (as planned in the protocol) was not implemented and the prespecified procedure was not fully followed. It was explained that due to few practical reasons, and to avoid multiple discrepancies to resolve while approaching multiple dermatologists-trichologists, it was decided to have a single-board member, i.e., one expert. Furthermore, the protocol-specified procedure dictated that investigator should the final decision when differing scores are to be reconciled. However, the multiple discrepancies that were detected between the investigators and the single expert's SALT evaluations led the applicant to seek the expert's opinion for a second time. The Applicant confirmed eventually that the investigator assessment of SALT was used in the FAS population (i.e., for the patients who had SALT score of 25-95 at Visit 1 by both the investigator and the expert). It remains unclear whether the decision to exclude subjects from the analysis population is associated with deficiencies in the protocol (e.g., the reconciliation process between investigator's and expert's SALT score assessment), or if the decisions during the study for defining the FAS were made as appropriate.

SALT≤20 was considered a clinically meaningful improvement for a patient with severe AA. For Olumiant, the primary endpoint was proportion of subjects that reached SALT≤20 at week 36 in adults with severe AA (EPAR EMA/572597/2022, 2022). The applicant states that the endpoint SALT≤20 is a clinically meaningful improvement for patients with severe AA, but this is not reflected in the primary efficacy endpoint 'relative change in SALT score' in the RAAINBOW study. In addition, a definition of moderate AA and a clinically meaningful improvement for a patient with moderate AA was not established for a centrally approved medicinal product in the EU. The relevance of SALT≤20 is not the same for a patient with a SALT score <50 compared to a patient with a SALT score >50. For 26 patients in the RAAINBOW study, the investigators rated the SALT scores at Visit 1 just above the inclusion threshold of SALT 25, while the independent expert rated the same patient below or far below SALT 25. This further reflects the difficulties of efficacy assessment in patients with lower range of the SALT score and highlights the questioning of the relevance of SALT≤20 as a clinically relevant improvement in these patients.

The planned total sample size of 102 subjects randomised was slightly exceeded, which is acceptable. However, while 87 subjects were planned to be included in FAS, only 62 were finally eligible for statistical analysis. Several issues were raised concerning the limitations of the FAS. The sample size calculation for the primary endpoint assumed between treatment difference of 24%, with a standard deviation of 32%.

Study subjects were randomised in a 2:1 ratio to receive either LH-8 or placebo. No stratification factors were used. It appears that some of the randomisation numbers are missing. Upon CHMP's request, the applicant provided the full randomisation list, which included randomisation numbers not used. Unclarity regarding 3 of the unused numbers remains; however, is not pursued due to other methodological issues of major concern.

Analysis populations: The FAS included randomised subjects with at least one administration of the study drug, and a baseline SALT score between 25 and 95. The definition of FAS is not acceptable as it drastically reduces the analysis population from the randomised study population; indeed, the FAS population, used for the efficacy analyses was substantially smaller compared to that of the ITT (all randomised subjects). Although the SALT score between 25 and 95 was an inclusion criterion at screening, this criterion was also

re-checked at baseline (up to 4 weeks later), which caused 7 subjects with screening SALT score between 25 and 95 to have SALT values outside the inclusion interval at the baseline assessment. These were judged as "wrongly included" according to the applicant and were excluded from the FAS. Furthermore, the protocol stated that a central board of certified dermatologists-trichologists would evaluate all pictures to assess SALT scores independently from the investigators' assessments, to verify SALT scores centrally. If the relative difference between these two assessments exceeded a predefined limit, a query was sent to the investigator for a final decision. However, it has been reported in the CSR that while investigator assessed scores were used in the statistical efficacy analyses, the baseline SALT scores centrally assessed by an independent expert were used to determine the FAS population. This is not in accordance with the protocol. The choice to use the central read of SALT scores to determine the FAS population was not sufficiently justified *prior* to the study conduct and is therefore understood as a deviation from the protocol. When using the central independent readings to determine eligibility of the subjects for FAS, there were additional 38 subjects excluded from the FAS. This high number of excluded subjects (45 out of 107 randomised) is alarming and not acceptable as it damages the reliability of the study. The applicant pointed to the deficiencies in the SALT scores assessment in subjects who do not have extensive hair loss, previously published in the literature. The description of the discrepancies observed between baseline SALT ratings made by the investigators and the expert were reiterated. The - as claimed by the applicant - wrongly included subjects were apparent in the data showing the clusters of subjects scoring just above SALT 25, or just below SALT 95, by the investigators, while the same subjects were excluded by the expert. It is worth noting that all recruiting investigators had discrepancy from the expert's rating on either SALT<25 or SALT>95, which is problematic for the study conduct. It can be acknowledged that enrolment of subjects into the study was seriously affected by the subjective nature of the SALT assessment and deficiencies in the study procedures around inclusion of subjects. Of importance, according to the protocol, the primary analysis was to be based on the ITT population. Definition of FAS, which did not follow the ITT principle, was added as a change from the protocol, and introduced in the SAP authored late during the study conduct. It can therefore not be excluded that the change of the primary analysis set was decided upon information obtained from the ongoing study. Also, allowing a single expert to select the subgroup of randomised subjects for inclusion in the FAS, was not part of the procedures described in the study protocol. The FAS was not prospectively defined in the protocol, and in practice, represents a subgroup of subjects selected by the single expert, which is not acceptable as primary analysis population. The issue of reduced primary analysis population from what was intended in the protocol (i.e., selecting a subgroup of about 58% of the ITT population for inclusion in the primary analysis set) damages credibility and cannot be accepted.

During the assessment, analyses of the population based on the screening SALT scores assessed by investigators and in line with the intention-to-treat principle were requested. The applicant performed these ITT-based analyses of the primary and the 1st key secondary endpoints using MMRM as specified in the SAP. The analyses of the 2nd key secondary endpoint (proportion of responders achieving at least a 40% relative reduction in SALT score after 24 weeks) were performed on the ITT population, as specified in the SAP. These did not indicate any statistically significant differences between LH-8 and placebo. Planned sensitivity analysis of responders achieving 30% and 50% relative reduction in SALT score was not presented. From the applicant's response to the CHMP's LoOI, it became apparent that the efficacy results submitted in the initial MAA were considered invalid and incorrect by the applicant, and the new preliminary results after re-analysis were presented as part of the final response. The re-analyses of the primary endpoint showed no significant differences between treatments in the ITT population (p=0.35).

Endpoint (assessment of SALT score): Use of central independent readings to determine FAS does not only limit the analysis population but also indicates a large discrepancy between investigator and central read,

which points to a high inter-reader variability of the SALT scores. Using the central reader to 'mend' the investigators assessments at screening and baseline to select the most relevant FAS indisputably questions the relevance of the investigators' assessed SALT scores used in the primary and secondary efficacy analyses, which points to a questionable reliability and validity of centrally and investigator assessed SALT scores in this study and adds to uncertainty of the results. It would appear, based both on the referenced data and the discrepancy issues observed in the RAAINBOW study, that the SALT measure does not perform adequately across the entire moderate to severe AA population. Considering that the discrepancy issues are acknowledged by the applicant only at baseline, it may be concluded that they represent a feature of a poor study conduct, rather than a consequence of an inadequate primary endpoint. Efficacy analysis based on the ITT population was not submitted in the initial dossier; this analysis was provided on request and did not show statistically significant difference between LH-8 and placebo.

Primary analysis: The primary endpoint was planned to be analysed using MMRM in combination with multiple imputation for the subjects who withdrew from the study without any SALT score under treatment. However, the primary and key secondary endpoint (the relative and absolute SALT change from baseline) were checked for normality *prior* to performing non-parametric analysis, and MMRM analysis was provided for information only. The CSR failed to present the analysis methods that were used and is in fact inaccurate. In the current results presentation for the primary and the first secondary endpoint (in the CSR), it is not clear which analysis method that was applied to obtain *p*-values.

Sensitivity analysis: This was planned, using an ANCOVA model and LOCF imputation for missing values, however, was not found in the dossier. The use of LOCF imputation (or BOCF for subjects with only baseline value available) may be questioned due to the imbalance between the treatment groups in amount of missing data, and due to the fact that the condition, although progressive, appears to fluctuate over time with both improvements and relapses. Consequently, it is likely that the unobserved data for subjects discontinuing due to lack of efficacy or AEs, would in general follow a trend similar to the untreated subjects instead of the same constant level as the last observed or baseline SALT score. Therefore, additional sensitivity ANCOVA analysis would be relevant when multiple imputation method is used with jump-to-placebo response approach for discontinuations. Other sensitivity analysis of interest would be the multiple imputation jump-to-placebo approach for all missing data. All missing data could have been summarised. However, considering the major issues raised, no further requests on sensitivity analyses are made.

Multiplicity: Hierarchical testing of the primary and the ordered 2 key secondary endpoints is acceptable. Type I error is considered controlled on 5% level (two-sided), provided that the MMRM analysis was performed as pre-specified in the SAP. However, a doubt is raised regarding the option to switch to another analysis method. The methods of statistical analyses should have been prespecified and not data driven. In this respect, the SAP was clear on using MMRM analysis for the primary endpoint (relative change in SALT score) and for the first secondary endpoint (absolute change in SALT score). However, the SAP also postulated an option to rank-transform SALT data and to switch from the MMRM model to a generalised linear model in case that the basic assumptions of MMRM were not met. The decision procedure concerning this option was not described and no details were given in the SAP or the clinical study protocol about the non-parametric analyses that the applicant chose to switch to, which violates the general principle of pre-specification of statistical analysis in clinical trials. Of additional concern is also the implication that the option to switch to another analysis method has on the overall Type I error rate which the applicant did not address. Thus, the results based on non-parametric analysis of the relative and absolute SALT change from baseline cannot be regarded as pre-specified and multiplicity controlled and are therefore not acceptable for potential approval of the medicinal product. Overall, based on the presented data, it cannot be excluded that the MMRM analysis results were known when deciding to approach GLIMMIX analysis for the FAS. The differing

results of the MMRM and GLIMMIX analyses indicate that the promoted primary analysis based on the FAS is not robust. With regards to the type I error, it is not controlled at 5% due to data-driven selection of analysis method based on normality of data.

Other findings: Since patient disposition was not clearly presented at the time of submission, the applicant provided a consort flow diagram of patient disposition. Treatment and study discontinuations were further summarised by reasons. Subgroup analysis was planned by several groups; however, the results were not presented. It is therefore not possible to evaluate consistency of the results across the studied population.

Efficacy data and additional analyses

The applicant initially presented that the primary endpoint of relative change of SALT score in LH-8 after 24 weeks of treatment group was 22.87% and in placebo group -8.00% in the FAS population (which was a subgroup of ITT comprised of only 58% of patients). There was an unclear and insufficient interpretation of the main results. The re-analyses of the primary endpoint showed no significant differences between treatments in the ITT population (p=0.35). The performed re-analysis in the FAS population resulted in a borderline nominal statistical significance (p=0.0488), see Table 12 below.

Table 12. Relative change from baseline in SALT score - FAS population

			LH-8 (N=42)	Placebo (N=20)
MMRM on raw data	Week 24	N	33	19
		Adjusted mean (SE)	15.47 (7.86)	-10.83 (10.86)
		Adjusted mean difference	26.31	
		95% CI	[0.14;52.47]	
		P-value (Significance)	0.049 (S)	

MMRM on relative change from baseline in SALT score
Fixed effects: Treatment, Week, Treatment-by-Week interaction, Baseline Severity
Unstructured covariance matrix
Estimations: restricted maximum likelihood (REML) method for parameters and Kenward-Roger method for degrees of freedom
Imputation method: pattern-mixture model for the patients with no post-baseline SALT score
SE: standard error, CI: confidence interval

The study has failed to show compelling and robust efficacy expected for single pivotal studies also in the FAS population. Furthermore, evidence of efficacy on result in a subgroup (i.e., FAS population) is not acceptable considering the result in the ITT population.

The applicant also submitted a table of concomitant treatments in the FAS population. Concomitant treatments were used in both the treatment and placebo groups including topical corticosteroids. Among them, one participant in the treatment group was using betamethasone dipropionate (0.5 mg) during 26 days of the treatment period. This is not in line with inclusion criteria of the study protocol. It is agreed by the applicant that SALT≤20 appears clinically relevant in patients with severe AA, which is not reflected in the primary endpoint. For the Olumiant MA, the primary endpoint proportion of patients with SALT≤20 at week 36 compared to placebo was met in two pivotal studies in patients with severe AA (including ~1200 patients). The applicant presented a *post-hoc* analysis of proportion of patients achieving SALT≤20 and SALT≤10. Considering the small FAS population, it is not possible conclude with certainty the outcome of such low number of patients in this *post-hoc* analysis from a single study, since patients with AA experience regrowth of hair during the course of the disease. In addition, a clinically meaningful improvement for a patient with moderate AA has not been established for a centrally approved medicinal product in the EU. The relevance of

SALT≤20 is not the same for a patient with moderate AA (SALT score 25-49) compared to a patient with severe AA (SALT score 50-95). For the phase 2/3 RAAINBOW study subgroup analysis, this would require a sufficient number of evaluable patients, currently considered lacking and hence, a clinically relevant result was not shown.

The applicant put forward further argumentation on clinical plausibility of a persistent effect of Cinainu. The discussion regarding the relevance of the studied mechanisms, as presented by the applicant, is mainly hypothetical with some details supported by references. However, it is also the applicant's view that percutaneous penetration study on human skin showed a limited penetration, and that minimal systemic absorption of LH-8 actives is expected to result from topical application on scalp skin. Therefore, as it appears unlikely that the concentration at target, allegedly at the luminal side of vascular endothelial cells, should be much higher than in plasma, it seems that the data previously presented for exposure of caffeine and theobromine, in relation to data for *in vitro* effect, does not support an effect at suggested clinical dose. In conclusion:

- in the single pivotal phase 2/3 RAAINBOW study of Cinainu in the AA indication in children and adolescents, clinical efficacy has not been established;

- the complex pathophysiology of AA is not completely understood. Therefore, the discussion regarding the relevance of the studied mechanisms, as presented by the applicant, is mainly hypothetical and thus not sufficient as an evidence of effect;

- there is no reliable, robust non-clinical data supporting an effect, in case one had been shown;

- the composition of the product intended to be marketed and its comparability to the batches used in clinical and non-clinical studies included in the dossier has not been established.

Since no further confirmatory conclusions are possible in a clinical trial where the primary null hypothesis cannot be rejected, it is considered unnecessary to discuss the outcome of the secondary endpoints in the RAAINBOW study.

2.6.7. Conclusions on the clinical efficacy

Clinical efficacy of Cinainu in the AA indication in children and adolescents is insufficiently substantiated and was not established, given that the level of evidence normally expected for a single pivotal study in a MAA was not achieved as required by the scientific guideline (https://www.ema.europa.eu/documents/scientific-guideline/points-consider-application-1meta-analyses-2one-pivotal-study_en.pdf). The fundamental requirements of documentation that would consist of adequate and well-controlled data of good quality from a sufficient number of patients demonstrating a positive benefit/risk in the intended population, at the intended dose, and manner of use is considered not fulfilled.

In addition, it became apparent as part of the applicant's response to the CHMP Day 180 LoOI that the efficacy results submitted in the initial MAA are considered invalid and incorrect by the applicant. New results, obtained after re-analysis, were presented as part of the applicant's response, but are not satisfactory to demonstrate convincing efficacy. The findings of the audit indicating critical deviations from the analyses prespecified in the SAP and from the established statistical principles for longitudinal studies, add uncertainty about the conduct, and data quality of the pivotal study.

Overall, the applicant has not demonstrated that the medicinal product now applied for corresponds to that tested in the RAAINBOW study. Consequently, statements about the clinical efficacy of the herbal medicinal

product cannot be derived from the RAAINBOW study. Clinical efficacy of the product in the applied-for indication in children and adolescents is not substantiated.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

The clinical development programme includes one phase 2/3 clinical study: Double-blind, vehicle-controlled, randomised, multi-centre study to evaluate the efficacy and safety of LH-8 cutaneous solution in children and adolescents with moderate to severe scalp alopecia areata (RAAINBOW). Safety data are available for 107 subjects (71 treatment group and 36 placebo group) from 4 to 18 years. Additionally, LH-8 was used in 91 adult healthy volunteers in phase I studies evaluating photosensitization, single dose toxicity and repeated dose toxicity. Patient exposure from studies of LH-8 in adult patients with AGA and from studies of CG 428 in adult patients with chemotherapy induced alopecia was provided, but without detailed safety information.

In addition, post-marketing safety information of CG 210 topical solution, that has been launched in 2011 as a cosmetic-status lotion and commercialised in several countries, was provided. A total of 614 subjects were exposed to LH-8 or CG 428 (a cutaneous solution containing a double dose of the active substance of LH-8) in either company-sponsored studies, company-partners sponsored studies or investigators-initiated studies for 2 months or more (398 to LH-8 and 130 to CG 428).

Patients' exposure and patients' characteristics in the RAAINBOW study are provided the Table 13 below.

Table 13. Patients' exposure and patients' characteristics in RAAINBOW study

			Total	LH-8	Placebo
ITT population (n)			107	71	36
Duration of treatment (in weeks)	mean		24	23	25
	median		24	24	25
	min		2	2	12
	max		39	39	38
Duration of treatment (n)	<8 weeks		1	1	0
	8-<16 weeks		11	8	3
	16-<24 weeks		12	8	4
Age groups (n)	2-<6 years		16	9	7
	6-<12 years		42	29	13
	12-<18 years		49	33	16
	Total		107	71	36
Weight (in kg)	mean		Weight was reported only in case of SAEs.		
	median				
	min				
	max				
Severity of disease as per investigator (n)	SALT 25-50 (moderate)		56	39	17
	SALT 50-95 (severe)		51	32	19
	Total		107	71	36
Relevant comorbidities (n)	Other autoimmune disorders	Atopic Dermatitis, Eczema	8	6	2
		Hashinoto's thyroidis	1	0	1
		Lichen Striatus	1	1	0
		Allergic rhinitis, Haye disease	5	5	0
		Juvenile rheuma	1	1	0
		Thyrodite d1 Hashimoto	1	1	0
	Metabolic disorders	Down syndrome, Hypothyroidism, Muscle and joint pain, Osetomyelitis right foot, Selen Deficiency	5	4	1
	Asthma	Asthma, Asthma bronchiale, Bronchities	7	4	3
	Allergy	Food allergy (Nuts, Apple)	3	2	1

			Total	LH-8	Placebo
	Genetic skin disorder	Palmoplanta hyperkeratosis congenital	1	0	1
	Vitamin Deficiency	Vitamin B1 deficiency, Vitamin B12 deficiency, Vitamin D deficiency, Vitamin D insufficiency, Zinc deficiency	7	3	4
	Acne		4	3	1
	Accident with hospitalization		1	0	1
	Algic Menorrhafia		1	1	0
	Circomision		1	1	0
	Cough		1	1	0
	Fungal Infection of Scalp		1	1	0
	Left testis lowering		1	1	0
	Lipoma surgery		1	1	0
	Surgical cure of strabismus		1	1	0
	Thalassemia minor		1	1	0
	Tibia fracture		1	0	1
	Unstable bladder		1	1	0
	Urticaria		1	1	0
	Conjunctivitis		1	0	1
	Varicella chicken pdl		1	1	0
	Wilfred disease		1	1	0

2.6.8.2. Adverse events

Overall, 104 AEs were observed, and out of those 96 adverse events were treatment emergent adverse events (TEAEs). Out of these, 9 were drug related and 3 TEAEs were severe (1 in the LH-8 arm and 2 in the placebo arm). By the time the trial was over, 81 TEAEs were recovered/resolved and 15 were ongoing, see Table 14 below.

Table 14. Overall Summary of Adverse Events by Treatment – Safety Population

Characteristics	LH-8 cutaneous solution (N=71) n % E	Placebo (N=36) n % E	Overall (N=107) n % E
Any AEs	28 (39.4%) 62	17 (47.2%) 42	45 (42.1%) 104
Any SAEs	0	1 (2.8%) 2	1 (0.9%) 2
Any TEAEs	28 (39.4%) 58	17 (47.2%) 38	45 (42.1%) 96
Drug-related TEAEs	4 (5.6%) 5	4 (11.1%) 4	8 (7.5%) 9
Severe TEAEs	1 (1.4%) 1	1 (2.8%) 2	2 (1.9%) 3
Serious TEAEs	0	1 (2.8%) 2	1 (0.9%) 2
TEAEs Leading to Study Drug Withdrawn	1 (1.4%) 1	0	1 (0.9%) 1
TEAEs Leading to Study Drug Interruption	1 (1.4%) 1	0	1 (0.9%) 1
TEAEs Leading to Death	0	0	0

[1] Percentages are computed using N provided in the Column header.

[2] AE: Adverse Event, TEAE: Treatment Emergent Adverse Event, n: Number of subjects; E- Number of Events

The list of Adverse Events per body system reported in the RAAINBOW study in provided in Table 15 below. Compared with AEs reported in the placebo group, none appeared specific or of special relevance. There has been one case of conjunctivitis in the LH-8 group and few cases of skin AEs were found in both groups. None were reported as severe.

Table 15. Organ Class (SOC) and Preferred Term (PT) – Safety Population

Characteristics	LH-8 cutaneous solution (N=71) n % E	Placebo (N=36) n % E	Overall (N=107) n % E
Total TEAEs	28 (39.4%) 58	17 (47.2%) 38	45 (42.1%) 96
Blood and lymphatic system disorders	1 (1.4%) 1	1 (2.8%) 2	2 (1.9%) 3
Lymph node palpable	0	1 (2.8%) 2	1 (0.9%) 2
Pseudolymphoma	1 (1.4%) 1	0	1 (0.9%) 1
Cardiac disorders, signs and symptoms NEC	0	1 (2.8%) 1	1 (0.9%) 1
Orthostatic intolerance	0	1 (2.8%) 1	1 (0.9%) 1
Ear and labyrinth disorders	0	1 (2.8%) 1	1 (0.9%) 1
Otitis media	0	1 (2.8%) 1	1 (0.9%) 1
Gastrointestinal disorders	2 (2.8%) 2	3 (8.3%) 4	5 (4.7%) 6
Abdominal pain	1 (1.4%) 1	0	1 (0.9%) 1
Abdominal pain upper	0	1 (2.8%) 1	1 (0.9%) 1
Aphthous ulcer	0	1 (2.8%) 1	1 (0.9%) 1
Diarrhoea	1 (1.4%) 1	1 (2.8%) 1	2 (1.9%) 2
Vomiting	0	1 (2.8%) 1	1 (0.9%) 1
General disorders and administration site conditions	6 (8.5%) 10	3 (8.3%) 6	9 (8.4%) 16
Pain	1 (1.4%) 1	0	1 (0.9%) 1
Pyrexia	6 (8.5%) 9	3 (8.3%) 6	9 (8.4%) 15
Immune system disorders	6 (8.5%) 6	2 (5.6%) 2	8 (7.5%) 8
Alopecia areata	4 (5.6%) 4	2 (5.6%) 2	6 (5.6%) 6
Conjunctivitis allergic	1 (1.4%) 1	0	1 (0.9%) 1
Skin reaction	1 (1.4%) 1	0	1 (0.9%) 1
Infections and infestations	9 (12.7%) 14	6 (16.7%) 8	15 (14.0%) 22
COVID-19	2 (2.8%) 2	0	2 (1.9%) 2
Dental Infection	1 (1.4%) 1	0	1 (0.9%) 1
Gastric and gastroenteric infections	2 (2.8%) 3	0	2 (1.9%) 3
Hordeolum	0	1 (2.8%) 1	1 (0.9%) 1
Lower respiratory tract infections NEC	0	1 (2.8%) 1	1 (0.9%) 1
Nasopharyngitis	4 (5.6%) 6	4 (11.1%) 5	8 (7.5%) 11
Pharyngitis	0	1 (2.8%) 1	1 (0.9%) 1
Tonsillitis	1 (1.4%) 1	0	1 (0.9%) 1
Tracheitis	1 (1.4%) 1	0	1 (0.9%) 1
Menstrual cycle and uterine bleeding disorders	1 (1.4%) 1	0	1 (0.9%) 1
Dysmenorrhea	1 (1.4%) 1	0	1 (0.9%) 1
Metabolism and nutrition disorders	1 (1.4%) 1	0	1 (0.9%) 1
Hypovitaminosis	1 (1.4%) 1	0	1 (0.9%) 1
Muscle disorders	2 (2.8%) 2	0	2 (1.9%) 2
Muscle strain	2 (2.8%) 2	0	2 (1.9%) 2
Musculoskeletal and connective tissue disorders	1 (1.4%) 1	0	1 (0.9%) 1
Musculoskeletal stiffness	1 (1.4%) 1	0	1 (0.9%) 1
Nervous system disorders	2 (2.8%) 2	1 (2.8%) 1	3 (2.8%) 3
Headache	2 (2.8%) 2	0	2 (1.9%) 2
Syncope	0	1 (2.8%) 1	1 (0.9%) 1
Psychiatric disorders	0	1 (2.8%) 1	1 (0.9%) 1
Anxiety	0	1 (2.8%) 1	1 (0.9%) 1
Reproductive system and breast disorders	0	1 (2.8%) 1	1 (0.9%) 1
Dysmenorrhoea	0	1 (2.8%) 1	1 (0.9%) 1

Characteristics	LH-8 cutaneous solution (N=71) n % E	Placebo (N=36) n % E	Overall (N=107) n % E
Respiratory, thoracic and mediastinal disorders	5 (7.0%) 6	3 (8.3%) 3	8 (7.5%) 9
Cough	2 (2.8%) 2	2 (5.6%) 2	4 (3.7%) 4
Influenza	2 (2.8%) 2	0	2 (1.9%) 2
Nasal polyps	1 (1.4%) 1	0	1 (0.9%) 1
Rhinitis allergic	0	1 (2.8%) 1	1 (0.9%) 1
Upper respiratory tract signs and symptoms	1 (1.4%) 1	0	1 (0.9%) 1
Skin and subcutaneous tissue disorders	9 (12.7%) 11	6 (16.7%) 8	15 (14.0%) 19
Acne	1 (1.4%) 1	2 (5.6%) 2	3 (2.8%) 3
Blister	1 (1.4%) 1	0	1 (0.9%) 1
Eczema	5 (7.0%) 5	3 (8.3%) 3	8 (7.5%) 8
Folliculitis	1 (1.4%) 1	0	1 (0.9%) 1
Pityriasis	0	1 (2.8%) 1	1 (0.9%) 1
Pruritis	1 (1.4%) 2	0	1 (0.9%) 2
Pruritus	0	1 (2.8%) 1	1 (0.9%) 1
Skin irritation	1 (1.4%) 1	1 (2.8%) 1	2 (1.9%) 2
Surgical and medical procedures	1 (1.4%) 1	0	1 (0.9%) 1
Tooth extraction	1 (1.4%) 1	0	1 (0.9%) 1

1. *Percentages are computed using N provided in the Column header.*
2. *AE: Adverse Event, TEAE: Treatment Emergent Adverse Event, n: Number of subjects; E: Number of Events*

Out of all the related TEAEs 7 were possibly related. The category of probably and definitely related had 1 TEAE each. Maximum of related TEAEs were due to the SOC of Skin and subcutaneous tissue disorders. Due to limited number of subjects and single reports of AEs, no specific safety pattern could be identified in the treatment group of this study. In the studies testing local tolerance and phototoxicity of the product, no phototoxic response was observed.

2.6.8.3. Serious adverse event/deaths/other significant events

Two serious TEAEs during the trial, both in the placebo arm, were reported, see Table 16 below. No serious AEs, deaths or other significant AEs with study product during the clinical development programme were reported.

Table 16. TEAEs observed in the RAAINBOW trial

Characteristics	LH-8 cutaneous solution (N=71) n % E	Placebo (N=36) n % E	Overall (N=107) n % E
Total TESAEs	0	1 (2.8%) 2	1 (0.9%) 2
Infections and infestations	0	1 (2.8%) 1	1 (0.9%) 1
Pharyngitis	0	1 (2.8%) 1	1 (0.9%) 1
Nervous system disorders	0	1 (2.8%) 1	1 (0.9%) 1
Syncope	0	1 (2.8%) 1	1 (0.9%) 1

[1] Percentages are computed using N provided in the Column header.
[2] AE: Adverse Event, TEAE: Treatment Emergent Adverse Event, n: Number of subjects; E: Number of Events

2.6.8.4. Laboratory findings

Not applicable.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.6. Safety in special populations

No specific information provided.

2.6.8.7. Immunological events

Not applicable.

2.6.8.8. Safety related to drug-drug interactions and other interactions

No specific drug interaction studies have been performed with the LH-8 cutaneous solution and the literature review did not permit to identify pharmacodynamic drug interaction related to the product's components.

2.6.8.9. Discontinuation due to adverse events

Twenty-nine patients withdrew from RAAINBOW study: 1 because of an AE, 4 because of lack of efficacy, and 25 for other reasons which are detailed in Table 17 below.

Table 17. Reasons for withdrawals

No.	Treatment	Reason of drop-out	Lack of efficacy	Adverse Event	Other			
					Withdrawal of assent/ consent	Lost to Follow-up	Non-compliance	Unknown
1	Placebo	Subject indicated to PI that no satisfactory results are observed from the trial medication. He withdrew consent from the trial.	X					
2	LH-8	No improvement in hair growth so no follow-up	X					
3	LH-8	No compliance Treatment stopped after 2 months because of non-efficacy	X					
4	Placebo	Withdrawal of consent due to deterioration of alopecia areata.	X					
5	LH-8	AE after which a continuation of treatment would constitute an unacceptably high risk for the subject. AE no. 01 -Acute eczema scalp and Face		X				
6	LH-8	A discussion was done with PI regarding the subject and consent was withdrawn			X			
7	LH-8	Site team was continuously following up with subject for her/his scheduled visit in May 2021 but was not responding and informed that subject did not want to continue in the study due to family reasons.			X			
8	LH-8	Subject's parent was not ready to allow their child to continue the trial.			X			

No.	Treatment	Reason of drop-out	Lack of efficacy	Adverse Event	Other			
					Withdrawal of assent/ consent	Lost to Follow-up	Non-compliance	Unknown
9	LH-8	Parentwanted to stop the trial because they feared that the questionnaires would have psychological impact on child			X			
10	LH-8	Lost to follow up.				X		
11	LH-8	Subject was lost to follow up				X		
12	LH-8	Missing data.						X
13	LH-8	Lotion not used. One application of Dermoval gel					X	
14	LH-8	No longer wishes to continue the study.			X			
15	Placebo	A discussion was done with PI regarding the subject and PI declared the subject lost to follow up				X		
16	Placebo	Withdrawal of consent			X			
17	Placebo	A discussion was done with PI regarding the subject and PI declared the subject lost to follow up				X		
18	Placebo	Lost to follow up				X		
19	Placebo	Unknown						X
20	LH-8	As discussion was done with PI regarding the subject and PI declared the subject lost to follow up				X		
21	Placebo	Withdrawal of consent -journey to study center too time-consuming.			X			
22	LH-8	Lost to follow up				X		
23	LH-8	Lost to follow-up				X		
24	Placebo	Parents withdrew assent/consent			X			
25	Placebo	Withdrawal of assent/consent			X			
26	LH-8	Wish for termination.			X			
27	LH-8	Lost to follow up				X		
28	LH-8	A discussion was done with PI regarding the subject and PI declared the subject lost to follow up				X		
29	Placebo	Multiple Telephonic contact done by Site CRC and consent withdrawn			X			
30	LH-8	Withdrawal of consent			X			
31	LH-8	Loss to follow up.				X		

One subject had TEAEs leading to study drug withdrawal and one had TEAEs leading to study drug interruption. Treatment with LH-8 was withdrawn due to serious severe scalp and face eczema in patient with atopic dermatitis. The treatment was interrupted due to eczema and due to non-serious mild skin irritation.

2.6.8.10. Post marketing experience

CG 210 topical solution was launched in 2011 as a cosmetic-status lotion and commercialised in a number of countries by the consumer healthcare division of several pharmaceutical firms. As of January 2023, more than 2.6 million units have been distributed. Table 18 below presents the number of adverse events collected through cosmeto-vigilance and reported since then.

Table 18. AEs reported in 2.6 million units of CG 210 commercialised as a cosmetic-status product

	Total	**2021 & 2022**	**2019 & 2020**	**2017 & 2018**	**2015 & 2016**	**2013 & 2014**
Number of AEs, including:	85	0	2	26	48	9
(A) Number of complaints linked to established SAEs	1	0	0	0	1	0

(B) Number of complaints linked to non-established AEs	0	0	0	0	0	0
(C) Number of complaints linked to established AEs	60	0	1	15	36	8
(D) Number of complaints linked to non-established AEs	21	0	1	11	8	1
(E) Number of complaints linked to established AEs caused by misuse	3	0	0	0	3	0

Description of undesirable effects (85 cases): (A): 1 case - swollen eyes, redness, lip swelling, heartburn, itching, rash, pain, the patient stopped her work for 1 week. Concomitant use with Minoxidil. Not enough information to attribute this case to CG210. (B): none. (C): 60 cases. Most of cases are not attributable because products were used in combination with Minoxidil (in these cases most of described AEs are well-known to be side effects of Minoxidil. (D): 21 cases: scaly, rash, buttons, vertigo, migraine, itching, oily hair or AEs which could not be described. (E): 3 cases of misuse: 1 use by oral route, 2 well-known cases of allergy to one of the ingredients (cocoa).

Table 19 below presents AEs reported with CG 210 cutaneous solution in adults, ranked according to frequency and the system organ class.

Table 19. AEs according to frequency and the system organ class

MedDRA	**Undesirable effects (frequency)**			
System Organ Class	**Very common >1/10**	**Common >1/100 to <1/10**	**Uncommon >1/1000 to <1/100**	**Rare >1/10000 to <1/1000 and very rare (<1/10000) or unknown**
Skin and subcutaneous tissue disorders		Scalp and face eczema* Scalp skin irritation* Acne*		

*There was no difference in incidence of adverse reactions between patients treated with the medicinal product or placebo

2.6.9. Discussion on clinical safety

The clinical safety includes data for the products LH-8, CG 210 and CG 428. It is stated that the LH-8 and CG 210 are the different codes of the same product, and CG 428 topical solution includes double concentration of the same constituents. However, no specification of the products was provided. From the clinical development programme for the applied indication, safety data are available for 107 subjects (71 treatment group and 36 placebo group) from 4 to 18 years. Additionally, LH-8 was used in 91 adult healthy volunteers in phase I studies evaluating photosensitisation, single and repeated dose toxicity. In addition, post-marketing safety information of CG 210 topical solution was provided.

The safety database may be considered sufficient to describe the safety profile of LH-8. However, the number of paediatric patients is rather limited which hinders characterisation of safety of the studied product. In addition, the study groups were unbalanced regarding the comorbidities (60% in treatment group *vs* 47% in placebo group), particularly autoimmune disorders (19% *vs* 8%) and metabolic disorders (6% *vs* 3%). The mean and median exposure in weeks was similar in both groups.

Infections and infestations, skin and subcutaneous tissue disorders and general disorders and administration site conditions were the most reported SOCs in the LH-8 group (19.7%, 15.5%, 14.1%, resp.) and were higher for placebo (22.2%, 22.2%, 16.7%, resp.). Pyrexia, nasopharyngitis and eczema were the most reported PTs in the LH-8 group (12.7%, 8.5%, 7.0%, resp.). The frequencies of these PTs were also higher in the placebo group (16.7%, 13.9%, 8.3% resp.). In total, no TEAEs appeared specific or of special relevance.

No significant difference in TEAEs is noted between age categories. Regarding gender, more TEAEs and drug related TEAEs are reported in females compared to males (57.7% *vs* 27.3% and 11.5% *vs* 3.6%, resp.). However, the limited sample size and the small number of AEs (drug relates TEAEs 6 in females and 2 in males) precludes any firm conclusion.

The list of AEs per body system reported in the RAAINBOW study does not show significant difference between the treatment and placebo groups. There was one case of conjunctivitis in the LH-8 group and few cases of skin AEs in both groups. None were severe. AEs with definite/possible/probable causality with study treatment/placebo were alopecia areata, acne, eczema, folliculitis and skin irritation. Alopecia areata was reported once in groups; eczema twice in treatment group and once in placebo group; one event of folliculitis was reported in treatment group; one case of skin irritation was reported in both treatment and placebo group. Due to the limited number of subjects and single reports of AEs, no specific safety pattern could be identified in the treatment group of this study.

Twenty-nine patients withdrew from RAAINBOW study: 0 because of an AE, 4 because of lack of efficacy, and 25 for other reasons. One subject had TEAEs leading to study drug withdrawn, and one subject had TEAEs leading to study drug interruption. The treatment with LH-8 was withdrawn due to serious severe scalp and face eczema in patient with concurrent atopic dermatitis. The treatment was interrupted in one subject due to eczema and due to non-serious mild skin irritation.

Post-marketing information on complains/AEs was provided for cosmetic product CG 210. Following the decision to develop the medicinal product for children, CG 210 was renamed to LH-8. Although CG 210 has been on the market for more than decade, information on the subject exposure, indications for use, is limited. The reported complaints/AEs are limited and of uncertain quality.

2.6.10. Conclusions on the clinical safety

It has not been shown that the product intended for marketing corresponds to that in RAAINBOW study. The clinical development of LH-8 included limited number of paediatric patients. Conclusions regarding the short- and long-term safety can hardly be drawn. Although data provided by the applicant are not always consistent between the various reports provided, no major safety issues were identified, but characterisation of the safety profile of LH-8 is not complete.

2.7. Risk Management Plan

The CHMP and PRAC, having considered the data submitted in the application were of the opinion that due to the negative benefit-risk, the risk management plan cannot be agreed at this stage.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

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

2.8.2. Periodic Safety Update Reports submission requirements

Not applicable.

2.9. Product information

In light of the negative opinion, a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.* However, in light of the negative opinion, a satisfactory package leaflet cannot be agreed at this stage.

3. Benefit-risk balance

3.1. Therapeutic context

3.1.1. Disease or condition

AA is an immune-mediated, inflammatory hair disease causing nonscarring hair loss in both paediatric and adult patients. AA is a fluctuating disease and spontaneously remission in AA patients is common, but such improvements become a lot less frequent when patients have been suffering from the condition for a longer period with several episodes of the disease.

3.1.2. Available therapies and unmet medical need

The medicinal product Olumiant/baricitinib is authorised in the for the treatment of severe AA in adults. In 2023, Litfulo (ritlecitinib) was authorised for the treatment of severe alopecia areata (SALT≥50%) in adults and adolescents 12 years of age and older (oral use). There are no approved medicinal products for treatment of AA in children or for moderate AA. Current treatment guidelines advise on topical corticosteroids for children under 12 years of age. There is an unmet medical need, in particular in children under 12 years of age.

3.1.3. Main clinical studies

The clinical efficacy data for support of the AA indication in children and adolescents is one single pivotal phase 2/3 RAAINBOW study. The RAAINBOW study was a randomised, double-blind, vehicle-controlled multicentre 24 weeks study in parallel groups with a 24 weeks off-treatment follow-up period. Except for a difference in applied quantity based on the size of the head, the RAINBOW study involved one dose. The primary efficacy endpoint was the relative change in SALT score after 24 weeks, which was based on a 4 pictures-based scoring system, where each side of the head is split into 4 quadrants, and scored by the investigator accordingly, and a SALT score of 100% consisting of full-baldness. A SALT score of ≥50 is considered severe AA. The applicant refers to a SALT score of 25-50 as considered to be a moderate AA. Several secondary endpoints were included in the study that were also based on the SALT score or reflected other aspects of AA.

Patients were recruited in sites located in Europe and in India. Male and female children and adolescents aged 2 to less than 18 years with active AA involving 25% to 95% of the scalp between 6 months and 3 years in duration were included in the study. A total of 107 patients were randomised. The sample size calculation was based on the primary efficacy endpoint.

3.2. Favourable effects

The applicant stated that after the last-patient-last-visit, it appeared that both patients with SALT scores <25 and >95 were incorrectly included in the trial. Hence, out of 107 randomised patients, the applicant refers to the results from the FAS population (n=62). Of these 62 subjects, 10 dropped out before the visit 3 at 24 weeks, of which 9 belonging to LH-8 group and 1 to placebo group leading to 52 subjects completing the 24-weeks treatment period, 33 in the LH-group and 19 in the placebo group. In the initial submission, for the FAS population in the RAAINBOW study using the GLIMMIX method for primary analysis (instead of the prespecified MMRM analysis), the applicant claimed that after 24 weeks of treatment, the primary endpoint was met. The relative change of SALT score in LH-8 group was 22.87% and in placebo group -8.00%. The 1st key secondary endpoint, the absolute change of SALT score in LH-8 group was 12.72, while the relative change of SALT score in placebo group was -0.77. The difference was statistically significant (p=0.0013). Considering dropouts as non-responders, the 2nd key secondary endpoint, proportion of subjects achieving at least a 40% relative reduction in SALT score, was 26.2% in the LH-8 *vs* 5% in the placebo group. The difference was statistically significant (p=0.0484). However, as part of the applicant's response to the CHMP Day 180 LoOI, these initial results are considered invalid and incorrect by the applicant, and the new preliminary results after re-analysis were presented. During the Oral Explanation held on 15th Oct 2024, the applicant stated that the new results are no longer considered preliminary. The re-analysis in the FAS population, the relative change of SALT score in LH-8 group was 15.47% and in placebo group -10.83%. The re-analysis resulted in borderline nominal statistical significance of the difference (p=0.0488 in MMRM analysis, and p=0.0475 in using rank transformation).

The first key secondary endpoint of the absolute change of SALT score in LH-8 group was in the re-analysis 9.33, while the absolute change of SALT score in placebo group was -2.23. The difference was not statistically significant (p=0.081). The second key secondary endpoint, proportion of subjects achieving at least a 40% relative reduction in SALT score, was in the LH-8 group 26.19% *vs* 5% in the placebo group (p=0.0488).

3.3. Uncertainties and limitations about favourable effects

There are several uncertainties and limitations about possible favourable effects of Cinainu in the treatment of moderate to severe AA in children and adolescents:

- It has not been sufficiently demonstrated that the produced batches of the product applied for correspond to the batches that were used in the single pivotal RAAINBOW study, which was carried out without the active substances being adequately characterised. The manufacturing of the active substances was changed later. Therefore, there are several quality related differences between the proposed product and that used in the RAAINBOW study and demonstrating that these correspond, is not possible anymore (as the clinical batches are expired).
- No dedicated pharmacodynamic studies were performed. The proof-of-concept of Cinainu in the treatment of AA in children and adolescents has not been convincingly presented by the applicant.
- Efficacy of Cinainu was not established in the ITT population. The primary analysis population was markedly reduced from that intended in the protocol of the RAAINBOW study. Only a subgroup of approximately 58% of the randomised subjects was claimed by the applicant to be eligible for the efficacy analysis. The ITT principle is thereby not followed. Basing evidence of efficacy on results in a subgroup (i.e., FAS population) is not acceptable considering the results in the ITT population. No further confirmatory conclusions are possible in a clinical trial where the primary null hypothesis cannot be rejected (ICH E 9 Statistical Principles for Clinical Trials).
- The FAS was not prospectively defined, and definition of FAS was added as a change from the protocol, introduced in the SAP, which was authored late during the study conduct. Therefore, it cannot be excluded that the critical change of the primary analysis set was decided upon information obtained while the study was ongoing.
- In practice, the FAS represents a subgroup of subjects selected by the single expert. Allowing a single expert to select the analysis population among the randomised subjects was not part of the study protocol procedures and introduces bias.
- The impact of dropouts (imbalanced between the treatment groups) on the efficacy results was not investigated. The high drop-out rate was justified by Covid lock-down measures, preventing the patients to come to the clinic for planned study visits. To keep as many subjects as possible in the study during the Covid-lockdowns, an amendment of the study protocol to allow home-pictures was put in place. However, only 1 patient dropped-out, from the placebo group.
- In milder forms of AA, the chance of a spontaneous recovery is increased in contrast to extensive AA. Although, AA is a progressive condition, the natural course of the disease is that spontaneously remission is expected in particular in patients with limited AA. Since the impact of the natural course of the disease is part of the evaluation of the clinical benefit of the treatment, it demands that the benefit in the treatment group compared to placebo group should be compelling and this was not demonstrated.
- The risk of unblinding due to the active ingredients' odour in the LH-8 treatment is not known. It is the applicant's view that the manufacturing process includes steps to captures the volatile and reactive sulphur compounds of onion, that the product contains only 1% of lemon, and the part of lemon the product contains is the least odorant part and that the dry powder of cocoa and guarana are odour-less. However, a proper test to substantiate this evaluation of the risk of unblinding due to the odour of all the active ingredients treatment is missing.

- The efficacy results submitted in the MAA were considered invalid and incorrect by the applicant, and hence, new results after re-analysis were presented as part of the applicant's response to the CHMP LoOI. However, findings from the audit add uncertainty about the trial conduct. The re-analyses of the primary endpoint showed no significant differences between treatments in the ITT population (p=0.35), and in the FAS population (which was a subgroup of ITT comprised of only 58% of patients) there was a borderline nominal statistical significance (p=0.0488). There was a borderline nominal statistical significance (p=0.0488). Hence, the study failed to show compelling and robust efficacy, as would be expected from a clinical programme based on a single pivotal study.

Overall, considering the above-mentioned limitations and deficiencies, the RAAINBOW study cannot be considered as a confirmatory trial for a MA.

3.4. Unfavourable effects

The clinical safety database for the applied indication consists of 107 subjects (71 treatment group and 36 placebo group) from 4 to less than 18 years. Additionally, LH-8 was used in 91 adult healthy volunteers in phase I studies evaluating photosensitisation, single dose toxicity and repeated dose toxicity. Post-marketing safety information of CG 210 topical solution, that was launched in 2011 as a cosmetic-status lotion was also provided. However, it is not known whether the composition of the products is the same, and in what indication and in which population the product was used. Therefore, this information is not supportive.

During the RAAINBOW study, 104 AEs (62 in treatment group and 42 in placebo group) were reported. Of these 96 were TEAEs. Only 9 TEAEs were reported as drug related: 5 in treatment groups and 4 in placebo groups. Two serious TEAEs were reported in placebo group. The list of AEs per body system reported in the RAAINBOW study does not show any significant difference between the AEs in treatment group compared with AEs in the placebo group. AEs with definite/possible/probable causal association with active study treatment/placebo were alopecia areata, acne, eczema, folliculitis and skin irritation. AA was reported once in both treatment and placebo group; eczema was reported twice in treatment and once in placebo group; one event of folliculitis was reported in treatment group; one case of skin irritation was reported in both treatment and placebo group. No serious TEAE was reported in the treatment group. Two serious TEAEs were reported in the placebo group: pharyngitis and syncope, both AEs regarded treatment unrelated. There were no important identified or important potential risks identified, that need further characterisation.

3.5. Uncertainties and limitations about unfavourable effects

There are several uncertainties and limitations about unfavourable effects of the product for the treatment of moderate to severe AA in children and adolescents, e.g.:

- The safety database for the applied indication is rather limited and the composition of the product is not sufficiently demonstrated to be comparable with the product now proposed to be manufactured.
- There is a lack of
    - kinetic studies proving negligible systemic exposure;
    - toxicological assessment of compounds present in relevant concentrations in the product;
    - non-clinical long-term repeat-dose studies with dermal application;
    - satisfactory studies regarding genotoxicity and carcinogenicity.

- The impact of the high concentration of ethanol in the product with particular focus on the skin properties of the youngest population is not clear.
- The risk of local pharmacodynamic interactions with other topical products is not described.
- Further information is required regarding risk assessment on nitrosamines. The overall conclusion of the applicant is that there is a risk of presence of nitrosamines. However, the root cause for this risk is not identified. The final risk assessment provided by the applicant is missing further assessment of an excipient, the acidic pH as well as an investigation of the formation of the detected nitrosamine NDELA. Monitoring of this nitrosamine has not been specified as control at release and stability testing.

3.6. Effects table

Table 20. Effects table for Cinainu in AA in children and adolescents with an active scalp AA that involved 25% to 95% of the scalp (as measured by SALT score)

Results as provided in the initial MAA submission, but at the end of assessment the primary endpoint and the 1st secondary endpoint were considered invalid and incorrect by the applicant

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
PEP: Relative change in SALT score after 24 weeks of treatment	Relative change in scalp alopecia areata severity scores (SALT) from baseline value to be assessed after 24 weeks of treatment. (Visual assessment and global standardised scalp photographs for SALT evaluation.)	%	+22.87	-8.00	P<0.0001 Confidence interval not provided in numbers. Uncertainties related to major quality differences between the medicinal product now applied for and the composition used in the RAAINBOW study, high number of excluded subjects (45 out of 107 randomised), concerns about the type-1 error control, the main efficacy results are unclearly and insufficiently presented.	RAAINBOW study
First key SEP: Absolute change in SALT score after 24 weeks of treatment	Absolute change in SALT score from baseline at the end of 24 weeks' treatment period		+12.72	-0.77	P=0.0013 Cinainu 12.72±23.08 Placebo -0.77±24.85 Same uncertainties as for PEP applies.	RAAINBOW study

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Second key SEP: Proportion of "responders" (subjects achieving at least a 40% relative reduction in SALT score after 24 weeks)	Proportion of the responders, i.e. subjects achieving at least a 40% relative reduction in SALT score from baseline at the end of 24 weeks' treatment period	%	26.2	5	P=0.0484 Same uncertainties as for PEP applies.	RAAINBOW study
Unfavourable Effects						
Pyrexia	Frequency in subjects experiencing AE	%	8.5	8.3	The frequency of AEs was similar in both groups, some even higher in placebo (vehicle) group.	
Alopecia areata		%	5.6	5.6		
Nasopharyngitis		%	5.6	11.1		
Eczema		%	7.0	8.3		

Results provided in the response to the CHMP Day 120 LoQ but at the end of assessment considered invalid and incorrect by the applicant

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

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
PEP: Relative change in SALT score after 24 weeks of treatment	Relative change in scalp alopecia areata severity scores (SALT) from baseline value to be assessed after 24 weeks of treatment. (Visual assessment and global standardised scalp photographs for SALT evaluation.)	%	19.56±53.77	-5.07±132.0	P= 0.7583 MMRM analysis ITT population.	RAAINBOW study
PEP: Relative change in SALT score after 24 weeks of treatment	Relative change in scalp alopecia areata severity scores (SALT) from baseline value to be assessed after 24 weeks of treatment. (Visual assessment and global standardised scalp photographs for SALT evaluation.)	%	22.87±44.32	-8.00±52.42	P= 0.0543 MMRM analysis FAS population	RAAINBOW study
First key SEP: Absolute change in SALT score after 24 weeks of treatment	Absolute change in SALT score from baseline at the end of 24 weeks' treatment period		8.83±21.36	6.71±24.42	P=0.6078 MMRM analysis for ITT population	RAAINBOW study

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Second key SEP: Proportion of "responders" (subjects achieving at least a 40% relative reduction in SALT score after 24 weeks)	Proportion of the responders, i.e. subjects achieving at least a 40% relative reduction in SALT score from baseline at the end of 24 weeks' treatment period	%	28.2 (95% CI 18.1; 40.1)	27.8 (95% CI (14.2; 45.2)	P=0.9660 (Chi-Square test) ITT population	RAAINBOW study
Unfavourable Effects – N/A						

The efficacy results submitted in the initial MAA and in the response to the CHMP Day 120 LoQ are considered invalid and incorrect by the applicant. The following re-analysis results have been presented as part of the applicant's response to the CHMP Day 180 LoOI:

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
PEP: Relative change in SALT score after 24 weeks of treatment	Relative change in scalp alopecia areata severity scores (SALT) from baseline value to be assessed after 24 weeks of treatment.	%	n=56 Adjusted mean (SE) 7.91 (12.68)	n=33 Adjusted mean (SE) -9.40 (16.10)	Adjusted mean difference +17.31 95% CI -18.65;53.28 P=0.345 ITT population Primary imputation method	RAAINBOW study
PEP: Relative change in SALT score after 24 weeks of treatment	Relative change in scalp alopecia areata severity scores (SALT) from baseline value to be assessed after 24 weeks of treatment.	%	n=33 Adjusted mean (SE) 15.47 (7.86)	n=19 Adjusted mean (SE) -10.83 (10.86)	Adjusted mean difference 26.31 95% CI 0.14;52.47 P=0.049 FAS population	RAAINBOW study

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
First key SEP: Absolute change in SALT score after 24 weeks of treatment	Absolute change in SALT score from baseline at the end of 24 weeks' treatment period		+9.33	-2.23	Difference between groups 11.55 P=0.081 FAS population	RAAINBOW study
Second key SEP: Proportion of "responders" (subjects achieving at least a 40% relative reduction in SALT score after 24 weeks)	Proportion of the responders, i.e. subjects achieving at least a 40% relative reduction in SALT score from baseline at the end of 24 weeks' treatment period	%	26.19	5	Difference between groups 21.2 percentage points P=0.0488 FAS population	RAAINBOW study
Unfavourable Effects – N/A						

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The applicant claims that Cinainu (LH-8), is a topical anti-inflammatory treatment that can stimulate hair growth in children and adolescents with AA. However, no dedicated pharmacodynamic studies have been performed to support this product in AA indication and there is no established data for AA in children and adolescents from 2 to less than 18 years of age in earlier phases of development. The proof-of-concept of LH-8 in the treatment of AA in children and adolescents was not established.

The clinical efficacy data to support this application is one phase 2/3 RAAINBOW study. Male and female children and adolescents aged 2 to less than 18 years with active AA involving 25% to 95% of the scalp with a duration of between 6 months and 3 years were included in the study. The applicant classifies patients with a SALT score between 50-95 as severe, and with a SALT score between 25-50 as moderate AA. A total of 107 patients were randomised to Cinainu or placebo. After the last-patient-last-visit, based on expert consultation, patients with SALT scores <25 and >95 were both incorrectly included in the trial. Hence, the population that was correctly included (according to the applicant) comprises of 62 subjects (FAS population).

As per the protocol, a board of certified dermatologists-trichologists should have determined the SALT score independently from the investigators, but this has not been implemented and hence, an opinion of a single expert was used to select the analysis population, which is not in line the protocol. The primary analysis

population was markedly reduced; only a subgroup of around 58% of the randomised subjects was claimed as eligible for efficacy analysis. The ITT principle was thereby not followed. Furthermore, the FAS was not prospectively defined in the protocol, but its definition was added as a change from the protocol, introduced in the SAP during the study conduct.

Data from the FAS population were analysed in several ways (GLIMMIX method instead of prespecified MMRM analysis). After 24 weeks of treatment, the primary endpoint relative change of SALT score in LH-8 group was 22.87% and in placebo group -8.00%. The difference was statistically significant ($p<0.0001$). However, a subsequent audit of the primary analysis uncovered critical deviations from the analyses prespecified in the SAP and established statistical principles, which add uncertainty about the study conduct and data quality. The re-analysis in the FAS population resulted in borderline nominal statistical significance ($p=0.0488$ in MMRM analysis, and $p=0.0475$ in using rank transformation). The study thus failed to show compelling and robust efficacy expected for single pivotal studies also in the FAS population, as discussed earlier in the clinical efficacy section.

The safety database for the applied indication is limited. Based on the AEs per body system reported in the RAAINBOW study, there seem to be no significant difference between the treatment and placebo group. In addition, the possible effects of the high concentration of ethanol in the product, with particular focus on the skin properties (eyes, abraded skin, mucous membranes) of the youngest population, were not addressed.

Importantly, the RAAINBOW study was carried out without the active substances being adequately characterised and since then, the manufacturing of the active substances was changed in such a way that one cannot draw conclusions concerning the safety/efficacy/quality of the product subject to the MAA and the product assessed in the RAAINBOW study. It could not be demonstrated that the medicinal product now applied for corresponds to what tested in the RAAINBOW study. Consequently, clinical data derived from this trial cannot be considered as supportive for the MA of the intended product.

3.7.2. Balance of benefits and risks

There are major deficiencies still remaining in the application of Cinainu. The product used in the pivotal clinical study was not sufficiently characterised. Its components (extracts of *Allium cepa/Citrus lemon*, *Paullinia cupana* and *Theobroma cacao*) are not comparable (with regards to composition and manufacturing process) with those in the product proposed for marketing. Consequently, it cannot be assumed that the newly manufactured finished medicinal product is of comparable quality to the clinical batches. The necessary quality, safety and efficacy requirements need to be met for the product being considered under the MAA. In the current MAA, data supporting quality, safety and efficacy has been submitted for a product which is different to the product in which the MA is being sought and further, using different manufacturing methods and processes. This is inadequate. Investigation of the clinical efficacy and safety of a developed product with characterised known quality should have been conducted. The proposed product's stability was not satisfactorily demonstrated, and analytical tools were not adequate to control the finished product and demonstrate stability. The proposed applicant's concepts are not supported by the regulatory EU framework for herbal medicinal products.

The clinical efficacy of Cinainu in the AA indication in children and adolescents is insufficiently substantiated and has not been established with the level of evidence normally expected for a single pivotal study in a MAA (https://www.ema.europa.eu/documents/scientific-guideline/points-consider-application-1meta-analyses-2one-pivotal-study_en.pdf). The RAAINBOW study cannot be considered as a confirmatory trial for a MA considering the deficiencies and limitations identified. The fundamental requirement for on documentation

that consists of adequate and well-controlled data of good quality from a sufficient number of patients demonstrating a positive benefit/risk in the intended population at the intended dose and manner of use is considered not fulfilled.

Furthermore, the non-clinical data package has major deficiencies, as no long-term dermal toxicity studies were performed, and there is limited knowledge of the product constituents and their local effect or transdermal passage. The genotoxicity package is not in agreement with ICH S2(R1). No carcinogenicity study was conducted, and the overall submitted information is insufficient for a weight of evidence approach, see ICH S2(R1).

Based on the entire evidence provided by the applicant, the CHMP did not consider that the major objections on the quality, non-clinical and clinical aspects were resolved. Therefore, it was concluded that the B/R balance of Cinainu is negative.

3.7.3. Additional considerations on the benefit-risk balance

Not applicable.

3.8. Conclusions

The overall benefit/risk balance of Cinainu is negative.

4. Recommendations

Based on the CHMP review of data on quality, safety and efficacy for Cinainu in the treatment of moderate to severe alopecia areata in children and adolescents (from 2 to 18 years of age), the CHMP considers by consensus that the quality, safety and efficacy of the above-mentioned medicinal product is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the marketing authorisation for the above-mentioned medicinal product. The CHMP considers that:

Quality:

- The comparability between the finished product proposed for marketing and the clinical batches studied in the single pivotal clinical study (RAAINBOW trial) was not demonstrated. In addition, the applicant could not demonstrate that the manufacturing process of clinical batches and intended commercial batches is comparable and can ensure a consistent product quality. Differences in the manufacture of clinical batches and intended commercial batches have been identified but not adequately justified by the applicant. Furthermore, there are significant differences in the pivotal attribute drug extract ratio (DER) for the guarana and cacao extracts between the batches used in the single pivotal clinical study and the batches proposed for marketing.
- Stability is not demonstrated for the proposed finished product. The bioassay proposed by the applicant cannot be considered an adequate analytical tool to demonstrate stability and cannot be used to establish a shelf-life of the finished product. Supportive data might be used in the stability studies, but the bioassay proposed cannot replace stability studies under ICH conditions.
- Major deficiencies regarding the quality control of all extracts and the finished product (acceptance criteria for several specification parameters are not agreed, comprehensive validation of relevant

analytical procedures was not provided, etc.) have been identified. The requirements of scientific guidelines regarding the development of medicinal products (i.e. Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/CHMP/CVMP/287539 /2005 Rev.1), Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/162241/2005 Rev. 3), Guideline on quality of herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/201116/2005 Rev. 3), as well as the following reflection paper: Reflection paper on markers used for quantitative and qualitative analysis of Herbal Medicinal Products and Traditional Herbal Medicinal Products (EMEA/HMPC/253629/2007)) have not been followed.

- The final results of the risk assessment of nitrosamines have not been submitted. The overall conclusion of the applicant is that there is a risk of presence of nitrosamines. However, the root cause for this risk is not identified. The final risk assessment provided by the applicant is missing further assessment of an excipient, the acidic pH as well as an investigation of the formation of the detected nitrosamine. Monitoring of this nitrosamine has not been specified as control at release and stability testing.

Non-clinical:

- The safety of the product for long-term use in the intended young patient population, was not sufficiently substantiated by non-clinical data. No long-term dermal toxicity studies have been performed, and there is limited knowledge of the constituents of the product and their local effect or transdermal passage. The genotoxicity package is not in agreement with the scientific guideline ICH S2(R1), no carcinogenicity study has been performed, and the overall submitted information is insufficient for a weight of evidence approach, see ICH S2(R1).
- Supporting information regarding the long-term safety cannot be derived from the RAAINBOW study, since comparability was not proven between the finished product proposed for marketing and the clinical batches studied in the single pivotal clinical study (RAAINBOW).

Clinical:

- Notwithstanding the fact that the comparability between the finished product proposed for marketing and the clinical batches studied in the single pivotal clinical study (RAAINBOW trial) was not demonstrated, efficacy of the product in the applied-for indication in children and adolescents is insufficiently substantiated. The FAS population, as formulated by the applicant, is not acceptable as primary analysis population. It does not follow the intention-to-treat principle, since a large proportion of the randomised subjects is excluded. The FAS was introduced as a change from the protocol at late stage during the study conduct. It is unclear whether the applicant's decision to exclude subjects from the analysis population are associated with deficiencies in the protocol in regard to the reconciliation process among investigator's and the expert's assessment of the SALT score. The rationale for this change is unclear. The entry criterion in the study protocol was explicit and correct only in including subjects into the study who had SALT scores between 25 and 95, which was checked by the investigator at two consecutive visits in accordance with the protocol. This indicates that the right subjects were included in the study from the very beginning. Notwithstanding the choice of primary analysis population, the re-analyses of the primary endpoint showed no significant differences between treatments in the ITT population (p=0.35). The corresponding re-

analysis in the FAS population resulted in a borderline nominal statistical significance (p=0.0488). Thus, the results are not considered as compelling.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and post-authorisation measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

New Active Substance status

Based on the CHMP review of the available data, the CHMP considers that: liquid ethanolic extract of *Allium cepa* (onion) fresh bulb and *Citrus limon* (lemon) fresh fruit / dry aqueous extract of *Paullinia cupana* (guarana) seed / dry hydroethanolic extract of *Theobroma cacao* (cocoa) seed are to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.