STATISTICAL ANALYSIS PLAN

Safety and efficacy assessment of deoxycholic acid (Embella®) injectable solution for the reduction of unwanted submental fat

NCT Number: NCT06509438

Date: November 20, 2022

STATISTICAL ANALYSIS PLAN

Name of Test Drug:	Embella®
Methodology:	Interventional, single-armed
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Sponsor Representatives:	Zist Orchid Pharmed Co.
Statistical Analysis Plan Date:	November 20, 2022
Statistical Analysis Plan Version:	Version 1.0

Contents

Participate timeline....4
Section 1: Administrative information....5
Title and Trial registration....5
Trial registration number....5
SAP Version (SAP version number with dates)....5
Section 2: Introduction....5
Objectives....5
Primary objective(s):....5
Secondary objective(s):....5
Section 3: Trial Methods....5
Trial design – description of trial design....5
Inclusion Criteria....6
Exclusion Criteria....6
Withdrawal of Subjects....6
Randomization....7
Sample size....7
Section 4: Outcome Variables....7
Endpoints....7
Primary Endpoint....7
Secondary Endpoints....8
Safety End point....8
Section 5: Statistical analysis....10
Patient Characteristics and Baseline Comparisons....10
Primary and Secondary Endpoint Analysis....10
Safety Analysis....10
List and describe each primary and secondary outcome including details of: methods used for assumptions to

be checked for statistical methods 11
Missing Data 11
Covariate Adjustment 11
Statistical Software 11

Participate timeline

Visit No	**0**	**1**	2	**3**	**4**	**5**	**6**
	Day 1		Week 4 (±2 days) (Injection 2)/ Follow-up of subjects with a satisfactory response who do not require further treatment	Week 8 (±4 days) (Injection 3)/ Follow-up of subjects with a satisfactory response who do not require further treatment	Week 12±4 day	Week 16±4 day (In subjects with 2 injections)	Week 20±4 day (In subjects with 3 injections)
Time	Baseline	Injection 1					
Screening	X						
eligibility control	X						
Consent	X						
Demographic data	X						
Injection		X	X	X			
Pain assessment		X	X	X			
Digital Photography	X		X	X	X	X	X
Measurement of submental fat	X		X	X	X	X	X
Patients' satisfaction			X	X	X	X	X
Physicians' assessment According to SMF	X		X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X

Section 1: Administrative information

Title and Trial registration

Evaluation of safety and efficacy assessment of deoxycholic acid (Embella®) (manufactured by Espad Darou Pharmed Company) for the reduction of unwanted submental fat

Trial registration number

IRCT20190210042676N29

SAP Version (SAP version number with dates)

Version: 1.0, Date: November 20, 2022

Section 2: Introduction

Objectives

Primary objective(s):

Safety and efficacy assessment of Embella® injectable solution in reducing the unwanted submental fat.

Secondary objective(s):

- Assessment of the efficacy of Embella® injectable solution, in improving the standard grading of submental fat (SMF)
- Assessment of the efficacy of Embella® injectable solution in reducing the diameter of submental fat
- Patient's satisfaction with the treatment process
- Pain assessment during the injection using a 11-point visual analogue scale
- Safety assessment of Embella® injectable solution

Section 3: Trial Methods

Trial design – description of trial design

It is a single group, before and after clinical study

Inclusion Criteria

- Age between 18 and 65 years old
- Individuals with any severity of unwanted submental fat
- Voluntarily signed the informed consent form and consented to the 3-month follow-up
- Willing to actively participate in the study and the scheduled visits

Exclusion Criteria

- Any previous interventions to treat SMF
- Anatomical features or previous trauma in the injection site which is liable to interfere with SMF evaluation or result in an aesthetically unacceptable outcome after treatment
- Evidence of any cause of submental enlargement other than SMF
- Patients with a body mass index (BMI) > 30 kg m^2
- Those undergoing or considering a weight-reduction program
- Patients with a history of sensitivity to any components of the study medication or topical or local anesthetics
- Patients with a history of dysphagia
- Any inflammation, active infection, unhealed wound, or tissue scar at injection site
- Those using anticoagulants or NSAID or other medications that increase the risk of coagulation disorders within 7 days before injection
- Unrealistic expectations
- Pregnant or lactating women
- Those undergoing low sodium diets like hypertensive patients

Withdrawal of Subjects

- The patients were allowed and free to refuse to cooperate with the study and follow-up whenever they want, but the researchers try to collect the maximum information, including the reasons for leaving the study and, if possible, the final results of the treatment.
- If a patient missed 2 or more consecutively scheduled visits, he/she was dismissed from the study.
- Occurrence of any of the exclusion criteria
- Occurrence of serious adverse event (SAE).

Randomization

Since this is a single-group study, randomization is not applicable.

Sample size

This study will be conducted in 20 eligible participants.

Section 4: Outcome Variables

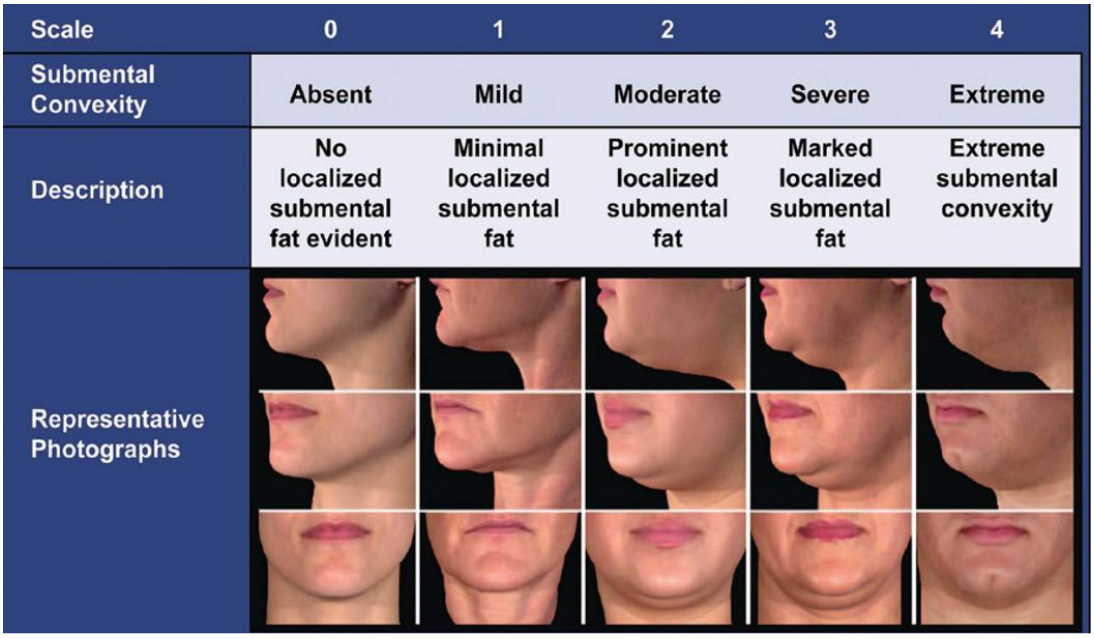
Endpoints

Primary and secondary endpoints are evaluated before injection and 4 and 12 weeks after the last injection.

Primary Endpoint

At least one-grade improvement in standard grading of submental fat (SMF) evaluated at 4 and 12 weeks after the last injection (Figure1).

Figure 1: Standard grading of submental fat (SMF)



Secondary Endpoints

- Submental fat diameter will be measured with the calliper at 4 and 12 weeks after the last injection
- Intensity of procedural pain will be assessed using the Visual Analogue Scale (VAS), ranges from 0 (complete painlessness) to 10 (maximum pain).
- Patient satisfaction will be assessed using the Visual Analogue Scale (VAS), ranges from 0 (not satisfied at all) to 10 (maximum satisfaction).
- Any observed or reported adverse events will be recorded after the injections and at 4- and 12-weeks follow-up visits, graded as mild [1] moderate [2] and severe [3].

Safety End point

Assessment of Adverse Events.

Adverse Event (AE) is described as any medical event that occurs in patients participating in a study, which differs from the clinical manifestations of the underlying disease progression and does not necessarily have a causal relationship with the treatment used in the study. Clinical manifestations that will be reported as adverse events include any symptoms, signs (such as any abnormal laboratory results), or temporary illness related to the use of the product being studied, whether or not these effects are causally related to the product under study.

Very common adverse events of DCA include:

- Pain
- Edema
- Numbness, tingling,
- Swelling
- Bruising
- Redness of the skin (erythema)
- Itching

Common adverse events of DCA include:

- Injection site reaction
- Bleeding
- Pain and discomfort

- Flushing
- Change of skin color
- Nerve injury around the jaw
- Skin tightness
- Difficulties in swallowing (dysphagia)
- Feeling sick (nausea)
- Headache

Uncommon adverse events of DCA include:

- Unusual taste in the mouth
- Difficulties in speaking
- Hair loss (alopecia)
- Hives (urticarial)
- Skin sores (ulcer)
- Scar

Adverse events of DCA where the frequency is not known include (frequency cannot be estimated from the available data) :

- Reduced or abnormal sensation in the area of the mouth (e.q. lip, tongue)
- Reduced sense of touch or altered sensation in the cheek
- Tissue damage and cell-death (necrosis) around the treatment area
- Injury of blood vessels if injected accidentally into artery or vein

The medical conditions that existed prior to the start of the study are only considered as adverse events if they have worsened during the course of the study and cannot be attributed to the natural progression of the disease.

Serious Adverse Reaction (SAR) is described as a harmful response to an intervention and includes:

•result in death.

•pose a risk of death.

•require hospitalization.

•prolong the duration of hospitalization.

•cause persistent or significant disability.

•result in congenital anomalies or birth defects.

• require medical or surgical intervention to prevent permanent damage

Section 5: Statistical analysis

Patient Characteristics and Baseline Comparisons

Basic characteristics are described using mean and standard deviation (SD) or median and range, frequency and percentages.

Primary and Secondary Endpoint Analysis

Descriptive analysis is performed using mean, SD or median and range, frequency and percentages. The efficacy of the treatment is assessed by comparing the mean/median value of each parameter among visits, using the Paired Sample T-test or the nonparametric equivalent, Wilcoxson test. The statistical significance level is defined as $p<0.05$.

Safety Analysis

All adverse events and adverse drug reactions will be recorded in the patient's medical file as well as in the designated section of the CRF. They will be classified based on severity, relevance, and relation to the treatment used in the study, and in accordance with the investigator's criteria and the guidelines outlined below.

Classification of adverse events based on severity:

Adverse events will be classified according to their severity into three categories: mild [1], moderate [2], and severe [3], based on the following definitions:

Mild: Any reaction, sign, or symptom that the individual can recognize but does not influence performance or functioning.

Moderate: Any reaction, sign, or symptom(s) of a sufficient severity to make the subject uncomfortable.

Performance of daily activities is influenced. This may require medical intervention.

Severe: Any reaction, sign, or symptom(s) of a sufficient severity to cause the subject severe discomfort. Performance of daily activities/functioning is compromised and causes disability and/or poses a specific risk to health. This usually requires medical intervention.

Note: Severe AEs are not necessarily serious unless one of the serious outcomes has occurred.

List and describe each primary and secondary outcome including details of: methods used for assumptions to be checked for statistical methods

For t-test, normality of distributions will be assessed using the Shapiro-Wilk test.

Missing Data

No imputation will be done.

Covariate Adjustment

No covariate adjustment will be done.

Statistical Software

The analysis will be carried out using SPSS software.