It was a randomised, double blinded phase II study evaluating the efficacy and safety of adipose tissue-derived mesenchymal stem cells versus placebo in patients with scarring or cutis laxa of the dorsal skin of the hands. The aim of the study was to demonstrate the safety and efficacy of autologous fresh or cryopreserved adipose tissue-derived stem cells delivered via topical injection in patients with scarring or cutis laxa of the dorsal surface of the hands. The active treatment arm A included 96 study patients (49 patients with cutis laxa and 46 patients with scarring). Arm B consisted of 20 patients according to the clinical treatment regimen. Patients in arm A received a dose of stem cell suspension isolated from lipoaspirate and then injected into the patient's skin. In arm B, placebo was administered to a hand or two scars or a single scar. The placebo was a 0.9% NaCl (saline) solution injected using the same procedure as for the stem cell suspension. Study arms A and B used a different cell application schedule:

Scheme I: 45 patients Arm A and 10 patients Arm B:
The first application of the cell suspension occurred on the day of adipose tissue collection by liposuction/lipoaspiration or 24h after collection. Patients received a fresh fraction of cells isolated from adipose tissue - not subjected to in vitro culture. This is the so-called stromal vascular fraction (SVF). This population, apart from mesenchymal stem cells (MSC), contains also CD31+ and CD34+ cells. Some SVF cells were frozen and some were prepared in cell culture for the second and subsequent applications in the patient. ADSC fraction cells, or MSCs isolated from adipose tissue, were applied in subsequent administrations.

Scheme II 49 patients Arm A and 10 patients Arm B:
The first application of the cell suspension took place 6-12 days after the liposuction/lipoaspiration procedure. This cell population was dominated by the MSC fraction (approximately 90% of the total population). Preparation of the cell suspension was preceded by cell culture lasting 6-12 days. The cells from the culture were used up to a maximum of 3 passages. Some cells were cryopreserved and after thawing prepared in cell culture for the second and subsequent applications in the patient. In subsequent administrations, cells of the ADSC fraction were applied.
The number of patients planned for inclusion was 100. The number of patients included in the study was 94.

Inclusion criteria:

1. Age 18 - 75 years at enrollment.
2. Informed consent signed.
3. female / male
4. scar or atrophic lesion of the skin on the back of the hands (cutis laxa)
a. Scar eligibility conditions:
Area:
o Abdomen
o Limbs
o Face
o Back
o Chest and neck
Time since creation: more than 6 months.
Scars not yet treated.
Atrophic and hypertrophic scars.
Two scars in a similar location, each between 1 and 6 cm and a total area between 1 cm2 and 5 cm2, or a single scar 2 to 16 cm long and an area between 1 and 5 cm2, or a marked fragment with an area between 1 and 5 cm2 for a scar with an area greater than 5 cm2.
Etiology:
o Post-traumatic

o Post-burn
o Surgical
b. Skin atrophic changes of the dorsum of the hands (cutis laxa)
Eligibility conditions:
- Extensive solar pigmentation
- Areas of pigmented lesions
- Sun spots
- Pigmented lesions also known as age spots.
- Rosacea
- Cracked capillaries
- Ruby birthmarks
- Atrophic changes of the skin and subcutaneous tissue
- Lesions symmetrically present on both hands
No previous aesthetic treatments in the area, previously standard
care.
6. patient's state of health that allows anaesthesia for liposuction.
7. willingness of the patient to attend follow-up appointments

Primary endpoint:
Time taken to see a 50% improvement in the patient's quality of life score from baseline.

Secondary enpoints:
1. Change in the volume of the fat layer at the application site assessed by change in skin and subcutaneous tissue thickness (ultrasound) and assessment of change in skin surface morphology by digital imaging.
2. change in tissue structure of the application site: surface area, circumference, tissue elasticity and thickness, colour and texture as assessed by the investigator and the patient
3. evaluation of the safety of the application method by assessment of adverse reactions.

Statistical methods used: Cox proportional hazards model, linear regression, logistic regression

RESULTS

EFFICACY ASSESSMENT:

Time to 50% improvement in quality of life was 22.6 weeks in the treatment group (95% CI 20.9 - 24.2) and 23.4 weeks in the placebo group (95% CI 20.3 - 26.4), with the difference not statistically significant by the log rank test (p=0.869). There was also a lack of statistical significance in the subgroups defined by diagnosis. In the subgroup of patients with scarring, the mean number of weeks to reach this endpoint was 22.3 (19.8 - 24.8) in the treatment group and 21.3 (15.3 - 27.3) in the placebo group (p=0.515). In the subgroup of patients with cutis laxa, these numbers were 22.8 (20.5 - 25.2) and 25.5 (25.5 - 25.5), respectively (p=0.334).
There were no differences between the treatment regimens evaluated. In the ADSC regimen, the mean number of weeks to endpoint was 22.2 (20.1 - 24.4) and in the SVF regimen it was 23.3 (21.3 - 25.2) (p=0.539). In the subgroup of patients with scarring, these numbers were 22.2 (19.0 - 25.4) and 22.0 (18.6 - 25.4), respectively (p=0.787), while in the subgroup of patients with cutis laxa, they were 22.3 (19.1 - 25.5) and 24.6 (22.6 - 26.6), respectively (p=0.522).

In univariate Cox proportional hazards models, there were no differences between the treatment and placebo groups in the overall study population (HR 0.925, 95% CI 0.347 - 2.488, p=0.877), neither in the subgroup of patients with scarring (HR 1.423, 95% CI 0.453 - 4.469, p=0.546) nor in the subgroup of patients with cutis laxa (HR 0.388, 95% CI 0.049 - 3.067, p=0.370). There were no differences between treatment regimens either; in the overall group the HR was 0.792 (95% CI 0.360 - 1.746, p=0.564), in the subgroup of patients with scarring the HR was 0.878 (95% CI 0.318 - 2.421, p=0.801) and in the subgroup of cutis laxa 0.673 (95% CI 0.190 - 2.387, p=0.540).
Also in multivariate Cox proportional hazards models, no statistical significance was found. In the overall group, the HR for the placebo vs drug comparison was 0.926 (95% CI 0.347 - 2.470, p=0.878) and for the comparison between regimens was 0.790 (0.358 - 1.741, p=0.558). In the subgroup of patients with scarring, the values were 1.410 (0.448 - 4.442, p=0.557) and 0.895 (0.324 - 2.476, p=0.832), respectively, and in the subgroup of patients with cutis laxa 0.389 (0.049 - 3.069, p=0.370) and 0.674 (0.190 - 2.389, p=0.541), respectively.
In terms of secondary endpoints, a significant difference was found in the investigator's assessment of distortion between the treatment arm and the placebo arm. The beta parameter in multivariate linear regression was 0.551 (p=0.018). No statistical significance was found for the other endpoints in the multivariate linear regression models.

SAFETY ASSESSMENT

A total of 150 AEs were reported in 56 patients in the study, including 13 severe, 48 moderate and 89 mild. At the Melitus centre, 27 AEs occurred in 10 patients, all of which were mild. At the Timeless centre 123 AEs occurred in 46 patients (13 severe, 48 moderate, 62 mild). One event at the Timeless centre resulted in withdrawal of drug product application: patient 2ABC02034 experienced "symptoms of general jitteriness" combined with an episode of elevated blood pressure (160/98). Adverse events related to the application procedure, were observed in 8 patients. In 95 of the 150 adverse events reported, treatment was recommended. None of the reported events were related to the study product. A serious adverse event occurred at the Timeless site in patient 2ABC01016, which was hospitalisation due to high blood pressure. The SAE ended with the patient recovering completely. In the investigator's assessment it was not related to the study product, but further administration of the product was discontinued. No deaths were reported in the study.

CONCLUSIONS
There was no clinical benefit on the endpoints assessed in the study population.

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