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CLINICAL STUDY REPORT (BRIEF VERSION)

AVUGANE

A double-blind, randomised, parallel group, placebo-controlled dose finding Phase II study to compare the efficacy and safety of topically applied Avugane™ of different concentrations in subjects with mild to moderate acne vulgaris

Study Title:
A double-blind, randomised, parallel group, placebo-controlled dose finding Phase II study to compare the efficacy and safety of topically applied Avugane™ of different concentrations in subjects with mild to moderate acne vulgaris

Protocol Number:
TT-AVUGANE™-Acne-02

Study Centres:
Two dermatological centres in Germany and one in Denmark

Study Duration:
Q1 2007-Q2 2008

Study Phase:
Phase II b

Objectives:
The primary objective of the study is to compare the efficacy of different concentrations of the study treatment Avugane™ on facial papulopustular acne in comparison to a placebo control.
The secondary objectives are to assess the safety, tolerability and the bioavailability of topically applied Avugane™ in different concentrations.

Methodology:
The study was carried out in three dermatological centres as a double-blind, randomised study with three active treatment groups of equal size (A – C) and a larger placebo group (D). In all treatment groups, in the evening, patients had to apply one of the following topical treatments:

Treatment group
1: Avugane™ 0.5%
2: Avugane™ 3%
3: Avugane™ 6%*
4: placebo

(* **Note**: By amendment group 3 was stopped after 6 patients due to problems with the formulation of the 6% medication).

Patients with mild to moderate facial papulopustular acne (acne comedonica or acne papulopustulosa) were to be enrolled in the study. After assessment and documentation of the baseline disease characteristics by the investigator, the patients were randomly assigned to one of

the above mentioned treatment groups and provided with study medication, containing one of the different concentrations of Avugane™ or placebo according to the randomisation. During the 12 weeks of treatment the patients were to apply one of the treatments to the affected facial skin daily in the evening.
Blood sampling for assessment of safety laboratory parameters were to be performed at the study sites on the day of screening and after 4, 8 and 12 weeks of treatment. Bioavailability to be tested pre- treatment (on day 1) and after 4 and 12 weeks of treatment. Efficacy evaluation to be performed at all scheduled visits at the study site. A follow-up visit to be performed 4 weeks after last application to monitor the treatment effects in comparison to the end of treatment.

Number of Subjects:
Twelve subjects were initially planned to be enrolled in each of the active treatment groups (1-3), and 15 subjects in the placebo group (4), resulting in a total of 51 subjects.
Note: Following an amendment recruitment to treatment with the 6% drug was stopped and planned patient enrolment was changed to **24** subjects in each of the active treatment groups (1-2), and **24** subjects **also** in the placebo group (4), resulting in a total of **72** subjects.

Diagnosis and Criteria for Inclusion:
Patients aged between 18 and 40 years with a papulopustular acne vulgaris (acne papulopustulosa or acne papulopustulosa et comedonica) with at least 12 inflammatory lesions on the facial skin appropriate for topical treatment.

The inclusion criteria are:
1. Clinically confirmed diagnosis of facial acne papulopustulosa or acne papulopustulosa et comedonica with a mild to moderate intensity (grade 2 to 8 according to the revised Leeds Scale of Acne Grade) and with at least 12 inflammatory lesions
2. Patients aged between 18 and 40
3. A general good health condition as confirmed by a physical examination and by medical history
4. Acceptance of oil-free skin care products
5. Acceptance to abstain from any other systemic or topical acne treatment during trial
6. Acceptance to abstain from sun bathing and the solarium
7. Signed informed consent

Exclusion criteria:
1. Females with positive pregnancy test at baseline
2. Females of childbearing potential without using a safe contraceptive measure (e.g. IUD or oral contraceptives, diaphragm or condom if used in combination with a spermicide), nursing women
3. Diagnosis of any acneiform diseases like exogenous acne, drug induced acne, cosmetic induced acne
4. Localisation of acne that requires treatment predominantly on the chest and/or the back
5. Patients with a personal or family history of cutaneous epithelioma
6. History or presence of disorders commonly accepted as contraindications to the treatment with 2-propylpentanoic acid (2-PPA) like history of any hepatic disease or jaundice, presence of any serious hepatic or pancreatic function disorders, severe renal function impairment, haematologic disorder like bleeding disorder or coagulation abnormalities, porphyria, bone marrow disorders, metabolic diseases (especially congenital enzymopathy), systemic lupus erythematosus or hypoproteinaemia
7. partial thromboplastin time (PTT) > 1.5 x UNL, serum creatinine > 1.5 x UNL, total bilirubin > 1.5 x UNL, liver transaminases (ALT/AST) > 2 x UNL
8. other skin conditions that might interfere with acne diagnosis and/or evaluation (such as facial psoriasis, seborrheic dermatitis, perioral dermatitis and papulo-pustular rosacea)
9. history of hypersensitivity to any of the substances in the test drug or in the placebo

10. treatment with any systemic acne drug within the last 3 months prior to the day 1 assessment and within 6 months for systemic retinoids
11. treatment with any other topical acne drug within the last 14 days prior to the day 1 assessment
12. presence of a significant uncontrolled cardiovascular, neurologic, malignant, psychiatric, respiratory or hypertensive disease
13. recent history of alcohol or any other substance abuse
14. treatment with any experimental drug within 30 days prior to the day 1 assessment
15. inability to comply with the study protocol

Test Product, Dose and Mode of Administration:
Avugane™ was provided as a clear off-white, semi-solid gel with different concentrations of the active compound (5 - 60 mg/ml 2-PPA). After washing the face with a mild cleanser, up to 1 g of the gel (amount will be defined by fingertip-units) will be applied daily to the clean and dry skin in the evening.

Duration of Treatment:
12 weeks

Reference Therapy and Placebo, Dose and Mode of Administration:
Placebo was provided as the base of the Avugane™ gel containing water, soy bean lecithin, phosphatidylglycerol, and 0.2 % sorbic acid.
The placebo gel, like the Avugane™ gels, was to be applied daily in the evening to the clean and dry skin after washing the face with a mild cleanser.

Criteria for Evaluation:
Efficacy
- Assessment of the therapeutic efficacy compared to the baseline will be evaluated by the investigator and by the subject according to a 7-point scale (must be conducted before counting lesions)
- Counting of lesions in the entire treated area of the face:
    A) Total count of acne lesions
    B) Separate count of non-inflammatory lesions (comedones)
    C) Separate count of inflammatory lesions (papules and pustules)
    D) Separate count of nodules
- Investigator's overall assessment of improvement by means of a photo documentation

Safety and Tolerability
- Safety laboratory parameters (haematology, clinical chemistry including coagulation tests, urinalysis)
- Clinical safety examinations (vital signs, physical examination)
- Adverse events
- Patient's assessment of local tolerability according to a 5-point scale

Pharmacokinetics
- Analysis of bioavailability of study drug by determination of serum concentrations of 2-PPA before start of therapy/day 1, and after 4 and 12 weeks of treatment.

Statistical Methods:
Demographic data will be summarised for all subjects by treatment using descriptive statistics (N, mean, standard deviation, minimum, median and maximum).

Primary endpoint:

efficacy: - change of total count of facial acne lesions from baseline to the end of week 12
- Change in lesions will be related to the drug concentration

Secondary endpoints

efficacy: - time to reduction of acne lesions by > 30%
- count of acne lesions in the face after 4, 8 and 12 weeks of treatment and at follow-up (4 weeks after the end of treatment) and its percentage and absolute change to baseline for the three parameters separately (all acne lesions, count of comedones, papules and pustules and total count of inflammatory lesions).

safety/tolerability: - safety laboratory parameters
- clinical safety (vital signs, adverse events)
- subject's assessment of local tolerability

bioavailability: - 2-PPA serum levels

The secondary end points will be related to drug concentration.

Primary and secondary endpoints will be evaluated descriptively including number of observations, mean, standard deviation, minimum, median, maximum and 95% confidence interval for the change during the therapy as well as for the original data (by study day). This will be related to the drug concentration. One way analysis of variance has been used for comparison of dose groups. Estimates of time to a 30% reduction in the number of lesions have been done using Kaplan-Meier statistics and comparison between the study doses have been done using the log-rank statistic.
Frequency tables will be generated for assessment of tolerability as it is an endpoint with ordinal qualitative outcome. This will be related to the drug concentration.

Adverse events will be summarised by a table including duration of event. This will be related to the drug concentration.
Descriptive statistics for laboratory parameters will be displayed by time of assessment, treatment and drug concentration.
Summary tables for 2-PPA serum concentrations will be displayed by time of assessment and drug concentration. Descriptive statistics will be calculated in order to provide more information about the penetration into the skin and the corresponding systemic absorption.

Results

Baseline characteristics and disposition

The target sample size was 72 patients, 70 patients were included from 3 centers (table 1a)

Table 1a. Disposition according to centre

Center	**N**	**Male**	**Female**	**Mean age**	**Randomized treatment group** 0.5%	3%	6%	placebo
1	23	6	17	25	8	7	1	7
2	21	4	17	25	6	6	2	7
3	26	20	6	23	6	8	3	8

Comparison of the randomized treatment groups demonstrated no significant differences in characteristics as gender, age and race and severity of the acne lesions (table 1 b)

Table 1 b. Baseline characteristics according to treatment group

BASELINE CHARACTERISTICS					
Treatment	**No of patients**	**Sex F/M**	**Total no. lesions Mean (range)**	**Inflammatory Lesions Mean (range)**	**Leeds score 2-8 Mean of grades**
0.5% Avugane	16	13/7	46 (21-118)	21 (8-45)	4.9
3.0% Avugane	18	11/10	49 (26-76)	24 (9-12)	5.0
6.0% Avugane	6	4/2	43 (21-57)	25 (14-51)	4.0
Placebo	16	12/10	47 (22-100)	24 (12-60)	4.7

Prior to the statistical analysis (Minutes from TT-Avugane Database Release meeting 03 Dec 2008) patients were grouped according to eligibility for inclusion in primary endpoint analysis (at least 42 days of treatment + d 85 assessment) (50 patients), secondary endpoints (at least assessment d 28 or d 85) (8 patients) or whether they were disqualified for efficacy analysis (few number of treatment days and no assessment (five patients) or major violation (one patient). Furthermore six patients had been randomized to treatment with 6% Avugane, an arm which was discontinued early in the study due to formulation problems. For regulatory reasons these six patients had to stop their treatment when the drug was withdrawn and they therefore only had from 18 to 37 days of treatment They are excluded from the primary endpoint analysis but for information included in some of the secondary analyses. The primary efficacy analysis (per protocol population) then had 50 patients for evaluation, 20 from centre 1, 16 from centre 2 and 14 from centre 3.

The primary endpoint

For each patient the investigators rating of the efficacy result was documented in the CRF and summarized 4 time-points in the study: d28, d56, d85 and at follow-up.

The primary endpoint is the change in the **total number of acne lesions** over the 12-week treatment period.

During the treatment the number of acne lesions decreased in almost all patients. Table 2 demonstrates for the three treatment groups the d85 mean count of acne lesions expressed as a fraction of the baseline value. a significant reduction is seen both in the total sum of acne lesions and in the subsets **inflammatory lesions** and **non-inflammatory lesions**. However, as detailed in the statistical report, there was no statistically significant differences in efficacy between the results from the three application methods neither in the change in total number of lesions or in the subsets.

Table 2. Mean change in acne lesions at 12 weeks (day 85)

		Mean change from baseline to day 85 counts of acne lesions (d 85 count as fraction of baseline count, + SD)

Treatment	No of patients	Total no. lesions	Inflammatory lesions	Non-inflam-matory lesions
0.5% Avugane	16	0.68 (0.26)	0.53 (0.26)	0.84 (0.60)
3.0% Avugane	18	0.64 (0.27)	0.71 (0.41)	0.65 (0.39)
Placebo	16	o.67 (0.36)	0.61 (0.25)	0.89 (1.23)

Secondary endpoints

When changes over the whole treatment period in the secondary endpoints are considered, table 2 demonstrates that for non-inflammatory lesions there is a trend towards higher efficacy in the 3% group, but the opposite for inflammatory lesions, and for neither of these groups can any statistically significant differences be demonstrated.
The protocol specifies a secondary end-point defined as the **time to a 30% reduction** in the number of lesions. The proportion reaching a 30% reduction was done using Kaplan-Meier estimates. For the total number of lesions, the Kaplan-Meier plots is shown in fig. 1 below.

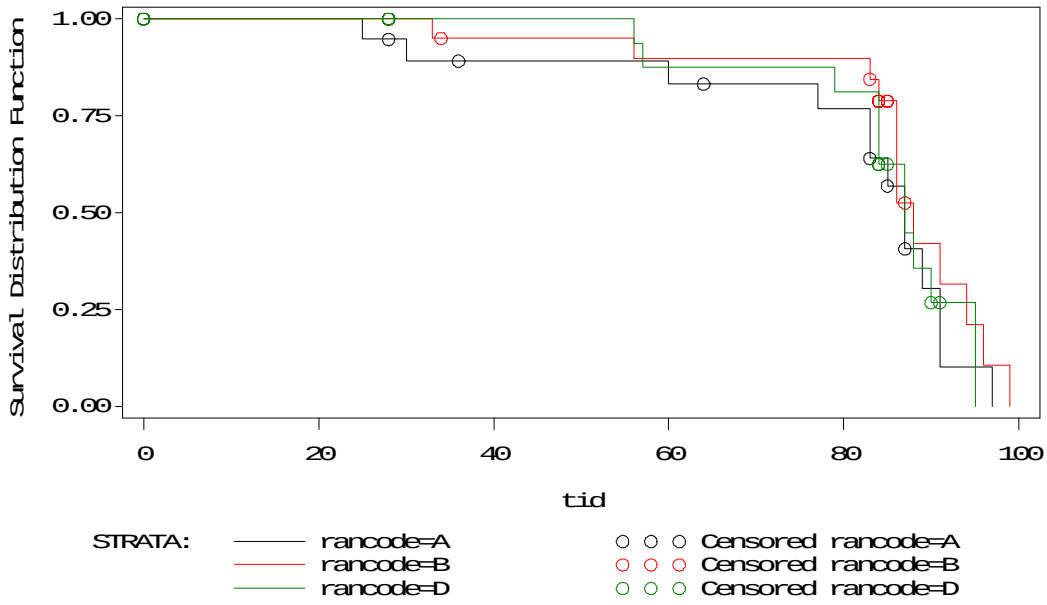


Fig.1. Time to a 30 % reduction for total number of lesions.

The graph in fig. 1 reflects the rather slow initial reduction in lesions but then a very steep increase in effect. No significant differences among the treatments were demonstrated in reaching the 30% reduction goal for the parameter total number of lesions or in corresponding analyses for the subsets inflammatory and non-inflammatory lesions.

The following tables demonstrate changes in acne lesions at **4 weeks** and at **8 weeks** following treatment start.

Table 3. Mean change in acne lesions at 4 weeks

		Mean change from baseline to day 28 counts of acne lesions (d 28 count as fraction of baseline count, + SD)		
Treatment	**No of patients**	**Total no. lesions**	**Inflammatory lesions**	**Non-inflam-matory lesions**
0.5% Avugane	18	0.83 (0.37)	0.80 (0.39)	0.89 (0.64)
3.0% Avugane	18	0.92 (0.27)	0.82 (0.32)	1.05 (0.42)
6.0% Avugane	6	0.81	0.77	1.01
Placebo	16	0.94 (0.42)	0.88 (0.40)	1.13 (1.04)

Table 4. Mean change in acne lesions at 8 weeks

		Mean change from baseline to day 56 counts of acne lesions (d 56 count as fraction of baseline count, + SD)		
Treatment	**No of patients**	**Total no. lesions**	**Inflammatory lesions**	**Non-inflam-matory lesions**
0.5% Avugane	16	0.68 (0.28)	0.73 (0.42)	0.67 (0.34)
3.0% Avugane	18	0.77 (0.34)	0.79 (0.34)	0.80 (0.45)
Placebo	16	0.80 (0.24)	0.83 (0.24)	0.79 (0.56)

The increasing efficacy with time is demonstrated when compared to table 2 and in the following two figures.

Fig.2 CHANGES WITH TIME- ALL LESIONS

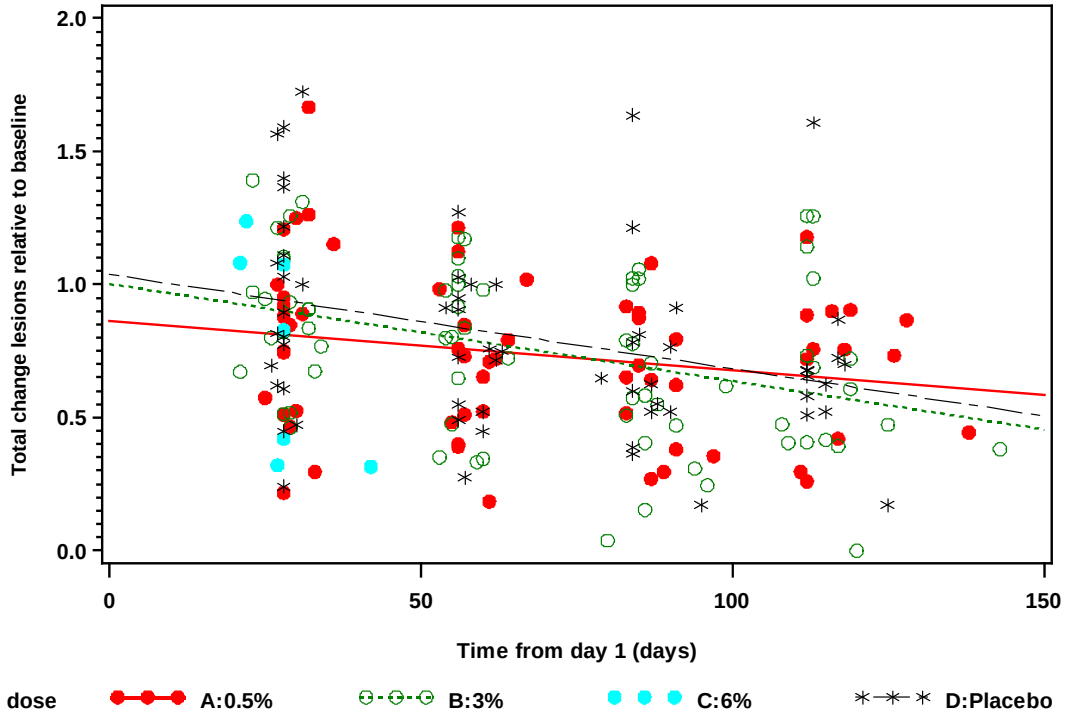


Fig. 3 CHANGES WITH TIME - INFLAMMATORY LESIONS

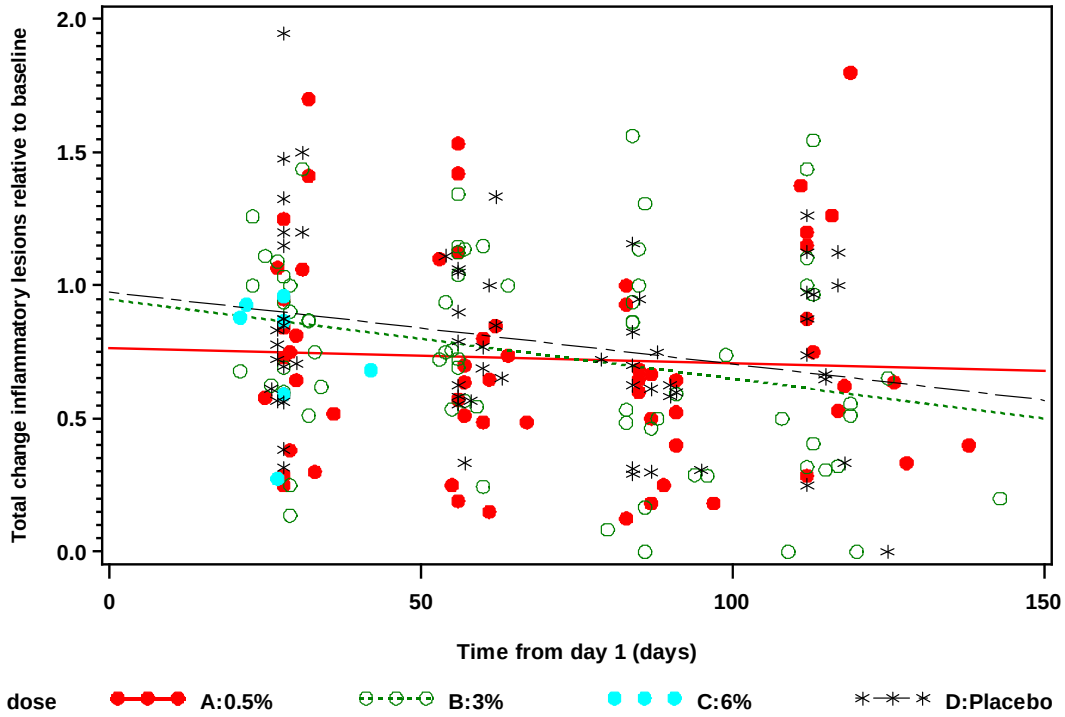
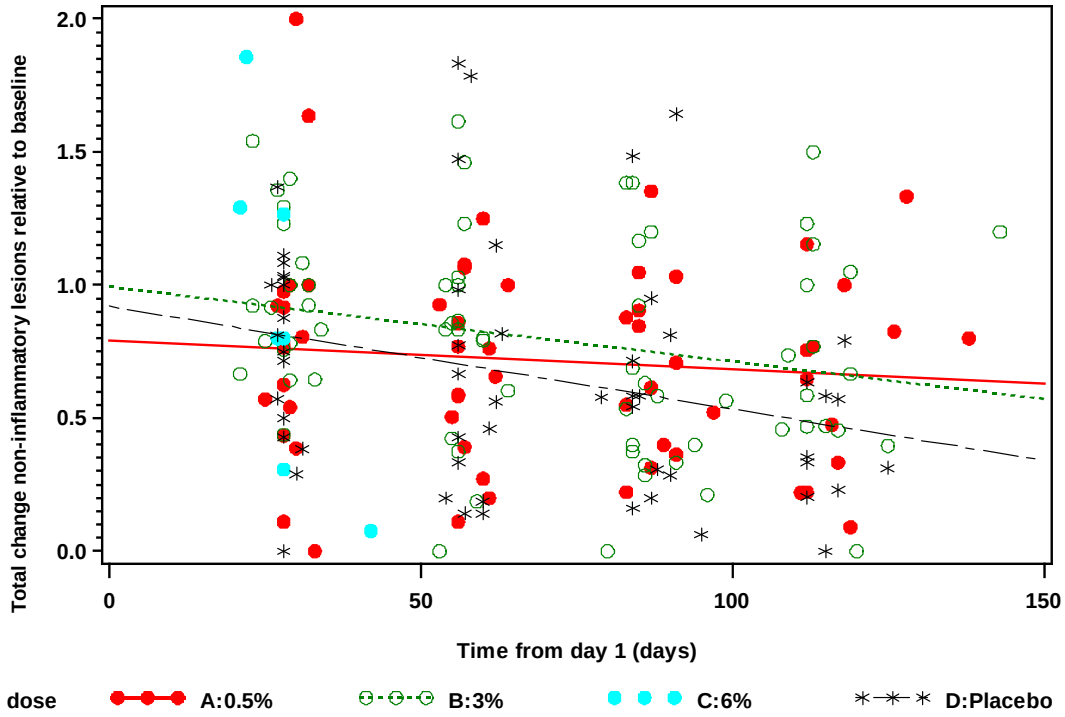


Fig. 4 CHANGES WITH TIME – NON- INFLAMMATORY LESIONS



The protocol asked for an **investigator overall assessment of therapeutic efficacy** at each of the 3 time points using 6 grades. The results are demonstrated in table 5 and show a trend towards more favourable outcome with time in the 3% group.

Table 5 Investigator assessment of efficacy at 3 time points

Group **n**	**0.5 %** **18**	**3 %** **19**	**6 %** **6**	**Plac** **18**	**0.5 %** **15**	**3 %** **17**	**Plac** **15**		**0.5 %** **16**	**3 %** **18**	**Plac** **16**
Evaluation Day	**28**				**56**				**85**		
Worse	0	1	1	0	2	0	3		1	1	0
No change	5	5	2	9	1	5	4		5	5	6
Minimal improvement	6	6	1	5	6	5	7		1	4	5

Moderate improvement	7	7	1		6	5	1		5	4	1
Marked improvement	0	0	1		0	1	0		4	3	4
Almost clear	0	0	0		0	1	0		0	1	0

Furthermore the patients were asked to make a similar evaluation of overall treatment effect at each of the 3 time points, table 6, again demonstrating a trend towards more favourable outcome for the 3% group with time.

Table 6 Patient assessment of efficacy at 3 time points

Group **n**	**0.5 %** **18**	**3 %** **19**	**6 %** **6**	**Plac** **18**	**0.5 %** **15**	**3 %** **17**	**Plac** **15**		**0.5 %** **16**	**3 %** **18**	**Plac** **16**
Evaluation Day	**28**				**56**				**85**		
Worse	2	1	1	2	2	1	4		2	0	1
No change	3	4	3	7	4	5	6		5	5	5
Minimal improvement	5	7	0	3	4	3	4		2	6	6
Moderate improvement	7	6	1	6	5	4	1		3	3	1
Marked improvement	1	1	1	0	0	2	0		4	3	3
Almost clear	0	0	0	0	0	1	0		0	1	0

Pharmacokinetics

As detailed in the pharmacokinetic report measurement of plasma concentrations of Avugane at baseline, on day 28 and on day 85 only demonstrated concentrations near the lower limit of detection with a range from 0.1 to 2.3 µg/ml, thus far below the 50 – 100 µg/ml concentration considered active in systemic therapy.

Safety

The treatment was generally well tolerated, all adverse events were rated as mild (grade 1) The patient assessment of tolerance is shown in table 5 below and the number of reported adverse events rated as related or possibly related to study treatment are shown in table 6.

Table 7. Patient assessment of tolerance

	Group			
	0.1%	**3%**	**6%**	**Placebo**
Acceptable tolerance	12	4	1	18
Well tolerated	19	19	1	18
Very well tolerated	14	27	4	13

Table 8. Adverse events (related or probably related). Note: all were of grade 1 only.

	Group			
AE	**0.1%**	**3%**	**6%**	**Placebo**
Dry skin	5	3	1	3
Redness	3	0	0	2
Burning sensation	3	1	0	0
Tenderness	2	0	0	1
Scaling	1	0	0	0
Itching	1	0	0	0
Eczema	1	0	0	0
Skin tension	0	1	0	0
Skin infection	0	1	0	0
Melasma	0	1	0	0
Finger tip rhagade	1	0	0	0

CONCLUSION

7o patients with facial acne were randomly allocated to a 12-week topical treatment with either Avugane 0.5%, Avugane 3%, Avugane 6% gel or a placebo gel. Early in the trial the 6% gel unfortunately showed stability problems and this arm of the study was discontinued, and the treatment of 6 patients already allocated was stopped after 18-37 days of treatment. The results from these 6 patients are nevertheless shown for information.

Patients had from 21 to 118 counted acne lesions (median 45) of which about half were of the inflammatory type.

The number of lesions (including subtypes) and the investigators and the patients overall impression of the treatment benefit was assessed after 4 weeks(d28), 8 weeks (d56) and 12 weeks (d85).

As demonstrated in tables 2, 3 and 4 and in the figures, the treatment induced a significant improvement over time both in the total number of acne lesions and in the subsets inflammatory or non-inflammatory lesions, an improvement which at d85 reached a 33-40% reduction. This was also born out by the investigator overall assessment at the time points (table 5) and the corresponding patient assessments (table 6).

Allthough the changes in the counts of the acne lesions did not reach statistically significant differences between the three treatment groups, the overall assessment both by the investigators and the patients did demonstrate a trend towards more favourable effect in the 3% group.

The original protocol had the 6% Avugane treatment as the target treatment and it was unfortunate that stability problems prevented a study of this probably optimal concentration. However the short treatment provided to six patients with the 6% Avugane did show promising

results particularly of the inflammatory lesions. All three grades of the 3% Avugane gels were very well tolerated.
The trend in the efficacy assessment does support a further trial of a new and more stable 6% Avugane.