FINAL REPORT

1) Study Title: Evaluation of topical cream containing cetylated fatty acids.

2) Study No.: 00-1076

3) Sponsor:

Imagenetix, Inc.
16935 W. Bernardo Dr.
San Diego, CA 92127

4) Principal Investigators

i) Immagenetix:

Dr. Robert Hesslink, Jr., Sc.D.

Telephone: 619 203-1609
FAX Number: 858 558-0662
Email: hesslinkjr@cs.com

ii) Perry Scientific:

Andrew W. Perry, M.D, Ph.D.
Perry Scientific, Inc.
7999 Vickers Street,
San Diego CA 92111

Telephone: (858) 467 9600
FAX: (858) 467 9600

5) Study Period: January 16, 2001 to June 18, 2001

6) Study Director Endorsement:

Andrew W. Perry, M.D., Ph.D.
Study Director
Date: 8-29-01

7) Introduction.

To this end a hairless mouse model was selected. Hairless mice have been widely used by the scientific community to evaluate dermal physiology and to study skin aging. Specifically the scientific literature indicates that chronological aging is marked by changes in the dermis and hypodermis as well as the collagen, adipose, and possibly proliferating cellular layers.[1]

8) Study Objectives

These studies were designed to assess Imagenetix's topical cream for:

i) eliciting skin irritation or rash;
ii) on cellular pathology;
iii) For its effect on skin aging.

9) Study Aims

i) To determine toxicity and safety of the topical cream per the following parameters,

   (1) H & E stain

ii) To determine the ability of the test article to prevent skin aging per the following parameters.

   (1) Morphology – Skin thickness

   (2) Connective tissue quantification

   (3) Cellular proliferation

10) STUDY DESIGN

Three time points were selected to represent various aging stages for the skin of maturing hairless mice. The three time points were day 10, day 58, and day 144.

Animals were sacrificed at each of the three time points to obtain a tissue biopsy for histological analysis. Each time point consisted of at least two groups, namely a treatment group and a placebo group as outlined in Table 1 and Table 2. For the last time point (day 144) a matched control was added as a third group. The matched control group consisted of tissue samples from the untreated opposing flank of each of the treated animals.

Table 1: Treated Group

Time Point	Dose/Schedule	Biopsies			Sac Day	n
[redacted]	[redacted]	Treated Site	Untreated site		[redacted]	[redacted]
I	50ul/ bid	yes	no		10	3
II	50 ul/ bid	yes	no		58	3
III	50 ul/bid	yes	yes		144	4

Table 2: Placebo Groups

Time Point	Dose/Schedule	Biopsies			Sac Day	n
[redacted]	[redacted]	Treated Site	Untreated site		[redacted]	[redacted]
I	50ul/ bid	yes	no		10	2
II	50 ul/ bid	yes	no		58	3
III	50 ul/bid	yes	no		144	2

11) MATERIALS AND METHODS

a) Animals

17 Hairless female mice 3 to 4 weeks of age, 20 –25 g body weight, from Simonsen Laboratories, Inc., Gilroy, California.

b) Model

This product requires evaluation using an *in vivo* model because its anticipated use will be in humans. The Hairless mouse model is the test system of choice because, historically, it has been used for similar studies and has been well characterized. It is also one of the lowest mammalian species with predictive clinical relevance to humans.

c) **Study Agents**

i) Test Article :

Imagenetix topical skin cream
Formula # N-18-088
Batch # RDN 18088
Storage: Room Temperature
Expiration Date: Not provided

ii) Placebo:

Water for irrigation, Baxter.

d) **Dose, Route, Schedule:**

(1) Dose: 50 µl of test article was applied per dose. A single concentration of the test article was used for all groups tested.

(2) Route: The topical method of administration of the test article is similar to the anticipated route of human exposure. The test article or placebo was topically applied to the right flank.

(3) Schedule: Twice daily. Morning and evening. Three groups of animals were dosed for 10, 58, and 144 days according to the study plan.

e) **Other Treatments or Procedures**

In order to accelerate skin aging the last set of animals biopsied were exposed to UV radiation for 10 days prior to sacrifice. The period of exposure was for 2 consecutive hours per day for 10 non-consecutive days. The animals received UV radiation for 5 weekdays, rested for 2 days and resumed treatment for an additional 5 days prior to being sacrificed.

f) **Endpoint:**

Animals from the treatment group and the placebo group were sacrificed at three predetermined time points. The animals were sacrificed in order to harvest skin for histological evaluation.

g) **Evaluation** – Skin biopsies were fixed in 10% buffered formalin. Fixed tissues were paraffin embedded, sectioned and stained by Hematoxilin and Eosin for morphological evaluation, Trichome histochemcial stain for collagen content evaluation, and Ki67 Immunohistological staining for cell proliferation evaluation. For those tissues sampled the stained sections were evaluated using a Computerized Image Analysis System to generate all or a subset of the following parameters: skin thickness, epidermis thickness, collagen layer thickness, and the epidermis cell proliferation index.

12) Result Summary Table

A) General - Mice behavior was normal and consistent with confined housing. There were no signs or symptoms of skin irritation as determined through visual inspection. Skin appearance was consistent with control mice without intervention.

B) Pathology – H & E stains were obtained for each data point (day 10, day 58 and day 144). There were no apparent abnormalities in cell lines.

C) Skin Thickness -

Placebo Group

	Day 10	Day 58	Day 144
Skin Thickness	232	249.62	442.05
Collagen Thickness	100.57	125.61	78.24
Epidermis Thickness	-	18.35	36.48
Proliferation	-	45.3	-

Treated Group

	Day 10	Day 58	Day 144
Skin Thickness	219.88	238.39	407.78
Collagen Thickness	115.39	126.44	85.46
Epidermis Thickness	-	22.81	34.23
Proliferation	-	40.83	-

Matched Control Group (untreated opposite flank of treated animal)

	Day 10	Day 58	Day 144
Skin Thickness	-	-	438.7
Collagen Thickness	-	-	88.74
Epidermis Thickness	-	-	34.7
Proliferation	-	-	-

13) Pathological Analysis:

The sponsor for this study requested no pathological analysis.

14) Conclusions:

Our observations indicate that as hairless mice age from weanlings to adults their skin changes from a thin translucent phenotype to a thicker and rougher state. This observation is supported by histological changes in the skin. Based on these observations it is expected that successful anti-aging treatments will result in prevention or the reduction of skin thickening as compared to controls.

In this study overall skin thickness compared to placebo animals was reduced between 5 and 8 percent. Indicating a possible benefit of test article in slowing down the thickening of the skin and potentially skin aging. This same effect was observed when the treated animals were compared to their Matched untreated controls (7%).

Collagen thickness was consistently higher in the treated groups as compared to the placebo groups. The significance of this finding is not clear.

Epidermal thickness demonstrated an increase of 24% on day 58 compared to placebo and a subsequent decrease of 6% compared to placebo at day 144. The significance of the initial increase is unclear however the subsequent decrease is consistent with the premise that a reduction in cellular layers may be associated with a reduction in skin aging.

Proliferation data was not sufficient to draw significant conclusions.

15) Recommendations:

Based on the results of this study the following two recommendations are made.

A similar study with larger cohorts of animals should be conducted to confirm the results of this study and reduce the margin of error.

A new study should be designed to test the ability of the test article to reverse or ameliorate the aging of skin in older animals. Preferably animals of advanced age in the range of 1 to 1.5 years.