CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-112

CLINICAL PHARMACOLOGY and BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS REVIEW

NDA: 21-112 **SUBMISSION DATES:** 3/19/99
PRODUCT: [redacted]
(fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%)
SPONSOR: Hill Dermaceuticals, Inc.
2650 S. Mellonville Ave., Sanford, FL 32773
TYPE OF SUBMISSION: Original **REVIEWER:** Sue-Chih Lee, Ph.D.

BACKGROUND

[redacted] Cream contains three active components: fluocinolone acetonide 0.01%, hydroquinone 4.0%, and tretinoin 0.05%. It is proposed for the treatment of cutaneous melanosis on skin phototypes II and III. The product is to be applied to the face and/or neck once a day before bedtime. No occlusive dressing is permitted. The sponsor conducted an in vitro percutaneous absorption study using dermatomed skin (thickness: 250-350 μm) from 3 donors. Clinical trials conducted by the sponsor compared the safety and efficacy of the proposed formulation to formulations containing any 2 of the three active ingredients.

COMMENTS

There are no in vivo studies to determine the systemic absorption or HPA axis suppression for the proposed formulation. Literature articles relating to systemic absorption of each of the three components were also provided. However, systemic absorption is greatly influenced by the formulation and manufacturing process. Thus, the literature data cannot be used in lieu of in vivo systemic absorption/HPA axis suppression studies.

RECOMMENDATION

There are no in vivo studies to determine the systemic absorption or HPA axis suppression for the proposed formulation. From the Clinical Pharmacology and Biopharmaceutics standpoint, the application is not acceptable.

[redacted]

Sue-Chih Lee, Ph.D.
Division of Pharmaceutical Evaluation III

RD/FT Initialed by Dennis Bashaw, Pharm.D. [redacted] 12/6/99

CC:
NDA 21-112
HFD-540 (Div.File)

HFD-540 (CSO/Lutwak)
HFD-880 (Bashaw)
HFD-880 (Lazor)
HFD-880 (Lee)
HFD-870 (attn: CDR. Barbara Murphy)
HFD-344 (Viswanathan)

Table of Contents:

I. FORMULATION

The proposed product is an oil-in-water formulation with the following components and composition:

Ingredient	% w/w
Tretinoin, USP	0.05
Fluocinolone acetonide	0.01
Hydroquinone	4.00
Magnesium Aluminum Silicate NF	
Butylated hydroxytoluene NF	
Cetyl Alcohol	
Stearic Acid	
Stearyl Alcohol	
Methyl Paraben	
Propyl Paraben	
Arlacel 165	
Methyl Gluceth-10	
Glycerin	
Citric Acid	
Sodium Metabisulfite	
Purified Water	
Total	100.00

II. IN VITRO PERCUTANEOUS ABSORPTION

Experimental

Cryopreserved skin from 3 human donors (6 samples per donor) was dermatomed to a thickness of approximately 250-350 µm and mounted onto ——— low-through diffusion cells with an exposure area of 63.6 mm^2 (9-mm diameter). The skin surface was maintained at 32°C and the receptor media was pumped through the chamber at a flow rate of 1.5 mL/hr. Each skin sample was tested for appropriate barrier function using tritiated water. ——— formulation (200 µL) was then applied to the skin surface.

Sample collections

Receptor fluid - collected at 1, 2, 4, 8, 12 and 24 hours which was extracted with ethyl acetate immediately after sample collection and stored at 4°C until reconstituted for HPLC analysis.

Skin samples - harvested and immediately processed for disposition analysis after 24 hours of exposure to the test formulation. ——— cream was removed from the skin samples with cotton swabs. Three of the 6 skin samples were washed twice with detergent and distilled water, and the washes were assayed for test article content. The other 3 skin samples were not washed. All skin samples were separated into component layers. The stratum corneum was removed by tape-stripping 5 times, and the epidermis and dermis were separated by heat treatment. Each of the skin layers was homogenized (1.8 mL deionized water and 200 µL 2N HCl). The samples were then extracted with ethyl acetate, dried and stored at 4°C until HPLC analysis.

Comment: Tretinoin is sensitive to light. There is no statement about lighting condition for the study.

Analytical

Comment: The validation results for the assay method was not provided. (Thus, this reviewer does not know the sensitivity of the method for either the receptor fluid samples or skin samples.)

Results

Flux of Active Ingredients into Receptor Fluid:
The flux of active ingredient from the cream through the skin sample and into the receptor fluid at various times following in vitro dermal exposure to the cream is given in Table 1. Hydroquinone was detectable in the receptor fluid with a flux plateaued at 1.5-5 µg/cm^2/h after 8 to 12 hours of exposure. After 24 hours of exposure, no detectable amount of fluocinolone or tretinoin was present in the receptor fluid collected from 2 of the 3 human skin donors evaluated. In the remaining one donor, small amount of fluocinolone (flux: ~9 ng/cm^2/h) or tretinoin (flux: ~0.8 ng/cm^2/h) was detected in 2 or 3 (out of 6) of the final 12-24 hr samples.

Washing of Skin Samples:
After 24 hours of exposure, the cream was removed from each skin surface with a cotton swab and half of the skin samples were washed to simulate a bathing session while the other half were not washed. The sponsor stated that the results between the two treatments did not differ but no data were provided. Thus, data from all samples were used to calculate cutaneous disposition.

Cutaneous Disposition:
Twenty-four hours after application of the cream to skin samples, both fluocinolone and hydroquinone could be detected in skin layers. The mean (±SE) concentrations in epidermis and

dermis were 9.4±1.1 µg/g and 2.3±0.2 µg/g, respectively, for fluocinolone and 2525 ± 1030 µg/g and 132±51 µg/g, respectively, for hydroquinone (Table 2). Tretinoin could not be detected in these skin layers.

Table 1: In Vitro Percutaneous Absorption – Flux (ng/cm²/h) at Various Sampling Times as Calculated from Receptor Fluid Data

Time (hours)	Fluocinolone	Hydroquinone	Tretinoin
Donor #1			
0-1	0.0 ± 0.0	33 ± 59	0.0 ± 0.0
1-2	0.0 ± 0.0	78 ± 35	0.0 ± 0.0
2-4	0.0 ± 0.0	239 ± 175	0.0 ± 0.0
4-8	0.0 ± 0.0	3043 ± 1367	0.0 ± 0.0
8-12	0.0 ± 0.0	2461 ± 1912	0.0 ± 0.0
12-24	9.3 ± 15.3	3052 ± 1661	0.0 ± 0.0
Donor #2			
0-1	0.0 ± 0.0	173 ± 180	0.0 ± 0.0
1-2	0.0 ± 0.0	1056 ± 1233	0.0 ± 0.0
2-4	0.0 ± 0.0	257 ± 92	0.0 ± 0.0
4-8	0.0 ± 0.0	5159 ± 4795	0.0 ± 0.0
8-12	0.0 ± 0.0	1824 ± 249	0.0 ± 0.0
12-24	0.0 ± 0.0	4683 ± 3754	0.8 ± 1.0
Donor #3			
0-1	0.0 ± 0.0	0 ± 0	0.0 ± 0.0
1-2	0.0 ± 0.0	68 ± 32	0.0 ± 0.0
2-4	0.0 ± 0.0	154 ± 135	0.0 ± 0.0
4-8	0.0 ± 0.0	403 ± 309	0.0 ± 0.0
8-12	0.0 ± 0.0	1026 ± 495	0.0 ± 0.0
12-24	0.0 ± 0.0	1455 ± 672	0.0 ± 0.0

Table 2: Mean (± SD) Epidermal and Dermal Concentration (µg/g skin) of fluocinolone, Hydroquinone and Tretinoin in Three Human Donors After 24 Hours of Exposure to Cream

Skin Layer	Fluocinolone	Hydroqunone	Tretinoin
Donor #1			
Epidermis	8.1 ± 2.0	2,307 ± 763	0.0 ± 0.0
Dermis	2.1 ± 0.2	73 ± 83	0.0 ± 0.0
Donor #2			
Epidermis	9.9 ± 12.7	1,621 ± 1,547	0.0 ± 0.0
Dermis	2.2 ± 0.4	168 ± 192	0.0 ± 0.0
Donor #3			
Epidermis	10.2 ± 3.6	3,647 ± 1,607	0.0 ± 0.0
Dermis	2.5 ± 0.7	154 ± 140	0.0 ± 0.0
Overall Mean (±SE) of 3 Donors			
Epidermis	9.4±1.1	2525 ± 1030	0.0 ± 0.0
Dermis	2.3±0.2	132±51	0.0 ± 0.0

Based on the quantity of active ingredient in each skin component after 24 hours of exposure, cutaneous disposition in terms of percentage of total amount recovered was computed (Table 3). For all three active ingredients, it was found that greater than 95% of the total recovery remained on the skin surface. For fluocinolone, approximately 4.3% of the total recovery was absorbed

with 1% in epidermis, 2.1% in dermis and 1.2% in receptor fluid. For hydroquinone, approximately 1.7% of the total recovery was absorbed with 0.3% in epidermis, 0.1% in dermis and 1.3% in receptor fluid. For tretinoin, less than 0.1% of the total recovery was absorbed with 0.03% in stratum corneum and 0.06% in receptor fluid.

Table 3: Cutaneous Disposition of Fluocinolone, Hydroquinone and Tretinoin in 3 Donors After 24 hrs of Exposure

Sample	Fluocinolone		Hydroquinone		Tretinoin	
	Mean ± SD (ng/cm^2)	% of Total	Mean ± SD (ng/cm^2)	% of Total	Mean ± SD (ng/cm^2)	% of Total
Donor #1						
Skin Surface	2,864 ± 681	92.18	3,526,095 ± 465,362	97.86	3,002 ± 1,742	99.93
Stratum Corneum	0.0 ± 0.0	0.0	855 ± 1,068	0.02	2 ± 4	0.07
Epidermis	46 ± 14	1.48	13,804 ± 6,445	0.38	0.0 ± 0.0	0.0
Dermis	86 ± 5	2.77	3,215 ± 3,830	0.09	0.0 ± 0.0	0.0
Receptor Fluid	111 ± 183	3.57	59,127 ± 24,115	1.64	0.0 ± 0.0	0.0
RF and Skin Layers	-	7.82	-	2.14	-	0.07
Total	3,107	100	3,603,096	100	3,004	100
Donor #2						
Skin Surface	4,874 ± 2,769	97.89	4,355,357 ± 2,076,365	97.86	5,092 ± 5,185	99.83
Stratum Corneum	0.0 ± 0.0	0.0	9 ± 13	0.0	0.0 ± 0.0	0.0
Epidermis	20 ± 19	0.4	3,510 ± 4,345	0.08	0.0 ± 0.0	0.0
Dermis	85 ± 15	1.71	7,359 ± 9,254	0.17	0.0 ± 0.0	0.0
Receptor Fluid	0.0 ± 0.0	0.0	84,564 ± 62,414	1.90	10 ± 12	0.17
RF and Skin Layers	-	2.11	-	2.14	-	0.17
Total	4,979	100	4,450,799	100	5,912	100
Donor #3						
Skin Surface	5,889 ± 2,460	96.95	5,301,887 ± 1,321,936	99.06	6,553 ± 4,856	99.97
Stratum Corneum	0.0 ± 0.0	0.0	46 ± 19	0.0	2 ± 3	0.03
Epidermis	70 ± 25	1.15	23,402 ± 5,263	0.44	0.0 ± 0.0	0.0
Dermis	115 ± 32	1.89	6,499 ± 5,243	0.12	0.0 ± 0.0	0.0
Receptor Fluid	0.0 ± 0.0	0.0	20,263 ± 2,597	0.38	0.0 ± 0.0	0.0
RF and Skin Layers	-	3.05	-	0.94	-	0.03
Total	6,074	100	5,352,097	100	6,555	100
Overall Mean (±SE) of 3 Donors						
Skin Surface	4542 ± 1540	95.7 ±3.1	-	98.3 ±0.7	-	99.9 ±0.07
Stratum Corneum	0.0 ± 0.0	0.0 ± 0.0	-	0.01 ±0.01	-	0.03 ±0.04
Epidermis	45 ± 25	1.01 ±0.55	-	0.30 ±0.19	-	0.0 ± 0.0
Dermis	95 ± 17	2.12 ±0.57	-	0.13 ±0.04	-	0.0 ± 0.0
Receptor Fluid	37 ± 64	1.19 ±2.06	-	1.31 ±0.81	-	0.06 ±0.10
RF and Skin Layers	-	4.33 ±3.06	-	1.74 ±0.69	-	0.09 ±0.07

Reviewer's Comments:

1. Regarding the experimental: The receptor fluid should be specified. Additionally, the amount of cream applied to the skin appeared to be a very thick layer (~300 mg/cm^2) which can maximize the amount absorbed but minimize the percentage absorbed.
2. Based on the receptor fluid data, in vitro percutaneous absorption in terms of flux was computed. It is more informative to add a plot of cumulative amount in the receptor fluid vs. time.

3. The sponsor indicated that 5 tape-strippings of skin samples represented the stratum corneum. In reality, stratum corneum may take more than 20 tape strippings. Thus, when looking at cutaneous disposition data, it should be noted that part of stratum corneum was included in the "epidermis."
4. The percentage of the active ingredients in various layers of skin or in the receptor fluid after 24 hours of exposure appeared to be calculated in terms of total recovery (Vol. 1.5, p. 34) and not based on total amount applied to the skin sample since all recovery adds up to 100%.
5. No or little tretinoin was found in the receptor fluid or stratum corneum 24 hours after application of the cream to skin samples. Since the lighting conditions during the study (tretinoin is light-sensitive) and assay sensitivity for tretinoin were not provided, the results cannot be put in a proper perspective. Similarly, the assay sensitivity for fluocinolone was not given.

Reviewer's Conclusion

The in vitro percutaneous absorption study indicated that the percentage absorbed for fluocinolone was actually higher than that for hydroquinone. However, the absolute value of % absorbed cannot be taken seriously as this is affected by the amount applied and amount recovered. In this study, the sponsor appeared to have applied a very thick layer of cream on skin samples which would tend to make the percentage absorbed appear lower. On the other hand, the % absorbed was computed based on total amount recovered and not based on the applied dose, which would make the percentage absorbed appear higher. Further, there is not a body of evidence to indicate that the in vitro percutaneous absorption is predictive of in vivo performance.

CLINICAL PHARMACOLOGY / BIOPHARMACEUTICS REVIEW

NDA Number:	21-112 (Amendment)
Submission Date:	07/20/01, 08/21/01, 10/25/01, 11/01/01, 11/21/01 and 11/22/01
Product:	TRI-LUMA™ (fluocinolone acetonide 0.01%, hydroquinone 4.00%, and tretinoin 0.05%) Cream
Sponsor:	Hill Dermaceuticals, Inc., Sanford, Florida 32773-9311
Reviewer:	Abimbola Adebowale Ph.D.
Type of Submission:	A response to the non-approvable (NA) letter (January 21, 2001) to the original NDA submission

Review of an Original NDA Amendment

I. Background and Introduction

This submission is an amendment in response to the non-approvable (NA) letter (dated January 21, 2000) issued to the original NDA (21-112) submission for TRI-LUMA™ cream. The proposed indication for TRI-LUMA™ cream is melasma of the face, and it is intended for once daily topical application. Melasma (also called chloasma, melanoderma) is a common acquired hyperpigmentation disorder characterized by irregular brown spots/patches on both sides of the face especially the lower cheeks, upper lips, nose, and chin. Melasma is seen primarily in women of childbearing age but can also occur in men.

The active ingredients of TRI-LUMA™ cream are fluocinolone acetonide, tretinoin, and hydroquinone. Fluocinolone acetonide, a corticosteroid, exerts an antimetabolic effect by decreasing epidermal turnover. This in turn may also affect the melanocyte by decreasing its secretory function. Hydroquinone, a depigmenting agent, interrupts one or more steps in the tyrosine-tyrosinase pathway of melanin synthesis. Tretinoin is a metabolite of Vitamin A that is classified as a keratolytic. It causes dispersion of pigment granules in keratinocytes, interferes with pigment transfer, and accelerates epidermal turnover, speeding up the loss of pigment.

In this submission the applicant included two clinical pharmacology studies to assess maximum systemic exposure after percutaneous absorption (Study #104479-70) and, to evaluate HPA axis (adrenal) suppression after 8 weeks of daily use (Study #33). The applicant stated that the contents of this amendment include the complete response to the deficiencies stated in the NA letter. In the NA letter there were two deficiencies related to human pharmacokinetics and biopharmaceutics as follows:

1. Under the subheading "Clinical/Statistical", item 3 was as follows "Studies on systemic absorption and HPA axis function (adrenal suppression) should be provided to support the systemic safety of the TRADENAME cream".
2. Under the subheading "Biopharmaceutics" the following was stated "Data should be provided from in vivo studies to determine the systemic absorption and HPA axis (adrenal) suppression for the proposed formulation".

A review of both studies is discussed below:

II. Adrenal Suppression Study in Patients with Melasma (Study # 33)

A. Study Design and Methods:

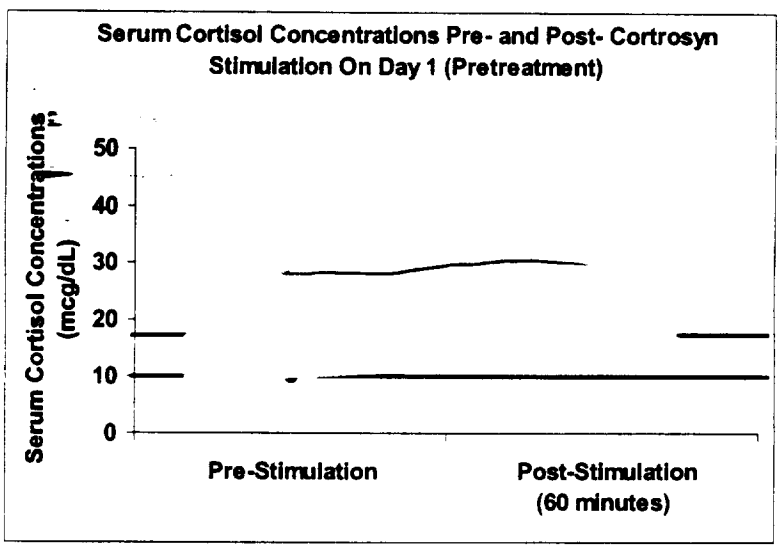
Details of the study design and methods are attached in the Appendix 1 (page 11–12). A brief summary of the study design is described here. This study was conducted as a controlled, open-label comparison involving 29 (27 females and 2 males, aged 27-68 years) patients with moderate (86.21%) and severe (13.79 %) melasma. Most patients (82.7%) were skin phototype II (always burns easily, tans minimally) or III (burns moderately, tans gradually). The patients received TRI-LUMA™ Cream once daily for a period of 8 weeks. The dosage was approximately 2 mg/cm^2 to the entire facial area for a total maximum exposure of approximately 360-mg.

In addition, patients were supplied with a cleanser (Cetaphil® Gentle Skin Cleanser), moisturizer (Cetaphil® Moisturizing Lotion), and a sunscreen (Presun®, Vanicream®, MDForte® or Neutrogena®) for daily use during the 8-week study. Blood samples for serum cortisol evaluations were drawn just before and 60 minutes after stimulation with 0.25 mg of Cosyntropin administered by intramuscular (IM) injection at pretreatment, Day 28, and Day 56. The applicant pre-specified criteria for a normal adrenal response was an 8-9 AM serum cortisol level of at least 10 µg/dL pre-stimulation and a serum cortisol level of at least 18 µg/dL approximately 60 minutes post stimulation. In the pre-NDA meeting on 06/16/01, it was recommended to the applicant by the Agency that the procedure and data analysis should be consistent with the information in the Cortrosyn® package insert as guidance. Although the procedure met this requirement the data analysis did not, probably because the applicant had already completed the study (Study dates 03/26/01 to 06/07/01) by the time the comments were conveyed to them.

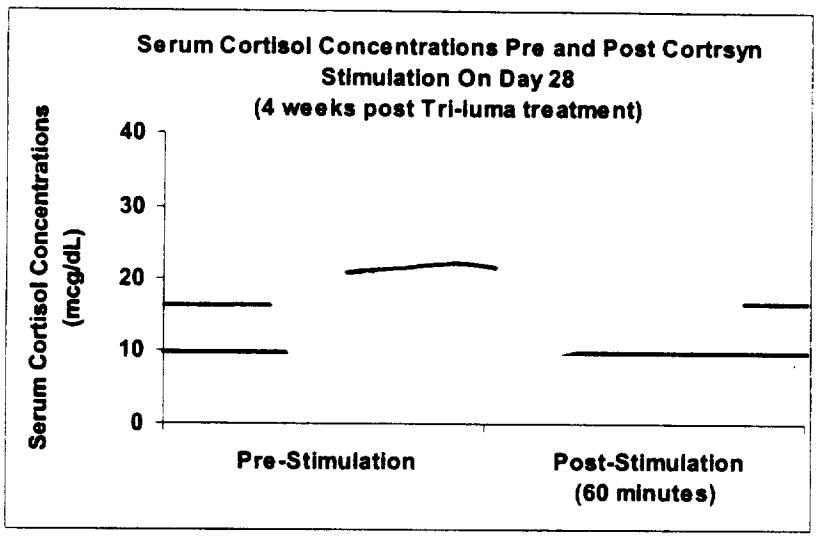
B. Results

1. Individual Plasma Cortisol Concentrations in Melasma Patients

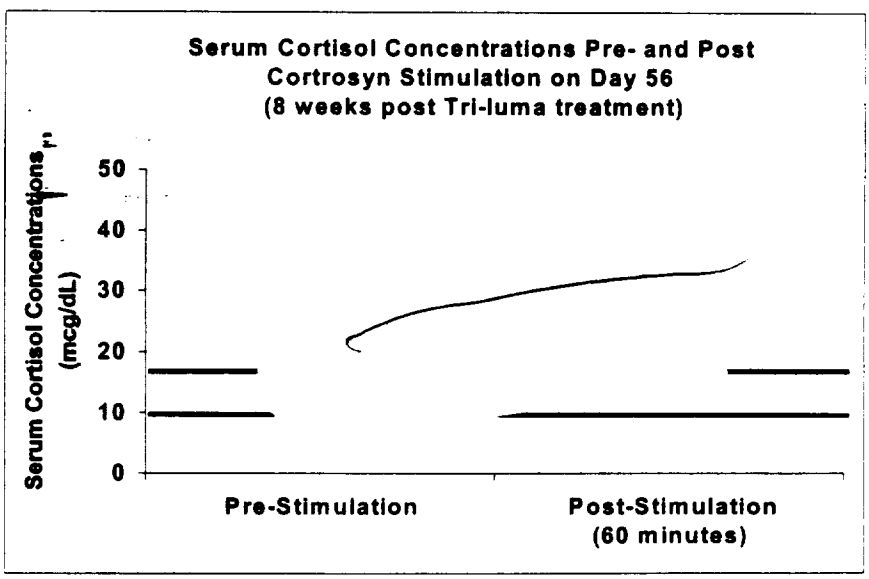
An evaluation of the individual plasma cortisol concentration data on Day 1 (pretreatment), Day 28 and Day 56 pre- and post-cosyntropin stimulation are reproduced in the graphs below. The individual plasma cortisol concentrations are attached in Appendix 1 (pages 13-15).



The above graph shows that on Day 1 (pretreatment) 28 patients met the applicant's pre-specified criteria representative of a normal response (i.e. pre-stimulation serum cortisol levels (>10 µg/dL). Patient # 14 had a pre-stimulation cortisol level value of [redacted] and should have been excluded from study entry, but the applicant included the patient. A possible explanation was that this value was within the laboratory reference AM range of 5-25 mcg/mL that denotes a normal response (although this was not a pre-specified criteria). However, all 29 patients had post-stimulation serum cortisol levels >18 µg/dL.



The above graph shows that on day 28, five patients (#'s 2,3,4,11,26) had pre-stimulation serum cortisol levels [redacted], and one patient (#3) had post-stimulation serum cortisol levels [redacted] However, this patient also had subnormal ([redacted]) pre-stimulation serum cortisol levels on day 28, suggesting that the patient was probably slightly suppressed.



The above graph shows that on day 56, one patient (# 4) had pre-stimulation serum cortisol levels [redacted] and all 28 patients had post-stimulation serum cortisol levels [redacted]. Applicant stated that one patient (#13) was not available on Day 56

The applicant did not include a pre-specified criteria for the 60-minute level increment over the basal value, however a general statement in the Cosyntropin package insert is as follows ... "If the 60-minute test period is used, the criterion for a normal response is an approximate doubling of the basal plasma cortisol value". An evaluation of the individual serum cortisol levels indicated that the number of patients that had an approximate doubling of the basal plasma level was 11/29 (37.9%) on Day 1 and 11/29 (37.9%) on Day 28 and 8/28 on Day 56 (28.6%).

3. ***Mean Plasma Cortisol Concentrations in Melasma Patients***

Inserted below are tables of the mean pre- and post-stimulation serum cortisol concentrations for Day 1 versus Day 28 and, Day 1 versus Day 56.

Table: Serum Cortisol Levels, Day 1 versus Day 28

	Cortisol Values (µg/dL) at		
	Day 1 Start of Study (N = 29)	After 4 Weeks of Treatment (N = 29)	Day 1 versus Day 28 p = value*
Cortisol Concentration at Pre-Stimulation†	16.7 ± 6.2	15.0 ± 5.1	0.024
95% CI	[redacted]	[redacted]	
Cortisol Concentration Post-Stimulation	29.6 ± 6.1	26.4 ± 4.6	< 0.001
95% CI	[redacted]	[redacted]	
Increase in Cortisol After Stimulation	12.8 ± 4.0	11.2 ± 3.8	0.061
95% CI	[redacted]	[redacted]	

*p-value from paired t-test

†Normal range for cortisol at 8 am = 5 – 25 µg/dL

The results in the table above indicate that there was a slight decrease in the mean serum cortisol concentrations after 4 weeks of treatment pre- and post stimulation and this difference was found to be statistically significant. However, the mean cortisol concentration at pre-stimulation and post-stimulation met the applicants pre-specified criteria (i.e. > 10 mcg/dL and > 18 mcg/dL). These results are consistent with the individual pre-stimulation serum cortisol concentrations obtained.

Table: Serum Cortisol Levels, Day 1 versus Day 56

	Cortisol Values (µg/dL) at		
	Day 1 Start of Study (N = 28)**	After 8 Weeks of Treatment (N = 28)**	Day1 versus Day 56 p = value*
Cortisol Concentration at Pre-stimulation†	16.6 ± 6.3	17.7 ± 6.3	0.348
95% CI			
Cortisol Concentration Post-Stimulation	29.7 ± 6.2	30.7 ± 7.2	0.269
95% CI			
Increase in Cortisol After Stimulation	13.0 ± 3.9	13.0 ± 4.6	0.986
95% CI			

*p-value from paired t-test

**Patient 13 not available for Day 56.

†Normal range for cortisol at 8 am is 5 – 25 µg/dL

The data in the table above indicate that comparisons of the pre- and post-stimulation serum cortisol concentrations on Day 1 and 56 were not statistically significant. Also following 56 days of treatment, the mean serum concentrations of cortisol pre stimulation were > than 10 mcg/dL and the mean serum concentrations of cortisol 60-minute post- stimulation were greater than the pre-specified value of 18 mcg/dL. One patient (# 3) on day 56 had a high pre-stimulation serum cortisol concentration ( ) and a low increase post stimulation to This patient also had a low response on Day 28 (pre-stimulation and mcg/dL post-stimulation). However, on Day 1 this same patient had a normal response (i.e. pre-stimulation and post-stimulation). The applicant stated that this anomalous Day 56 observation is probably due to a laboratory error.

C. Study Conclusions

The data demonstrated that after up to 56 days of exposure to TRI-LUMA™ cream suppression of the hypothalamo-pituitary-adrenal (HPA) axis was not observed based on the applicants' pre-specified criterion. Although the results on day 28 appear contradictory because five patients had low pre-stimulation levels, but only one of them ended up with slightly lower post-stimulation cortisol level. Therefore, any observed adrenal suppression is minimal and not likely to be clinically significant.

III. In Vivo Study on Systemic Absorption after Maximum Exposure to the Drug (Study # 104479-70)

A. Study Design and Methods:

The objective of this study was to determine the maximal systemic exposure, via percutaneous absorption under clinical use conditions for safety assessment. Details of

the study design and methods are attached in Appendix 2 (page 16). A brief summary of the study design is described here. This was a single-center, open-label, Phase I study with two groups (I and II) applying two different doses (1G and 6G). A total of 59 subjects were enrolled in both groups with forty-five subjects (5M, 40F) in Group I and fourteen subjects (2M, 12F) in Group II. Subjects in Group I applied approximately 1 gram of cream to cover the left or right forearm (between the wrist and elbow) once daily for 8 weeks. Subjects in Group II applied approximately 3 grams of cream to cover each forearm (between the wrist and elbow) simultaneously, daily for the eight week duration of the study.

Blood samples were collected on Days 1, 7 and 14 at 0, 2, 4, 6, 8, 12 and 24 hours post dose. Additionally, blood samples were obtained on Days 4, 21, 35 and 56 prior to treatment. Plasma samples were tested for hydroquinone, tretinoin and fluocinolone acetonide levels.

B. Results

1. Analytical Method and Validation

A brief summary of the analytical methods and their validation are described below. Details are attached in Appendix 3 (pages18 and 19)

Hydroquinone:

Fluocinolone acetonide: Method 1:

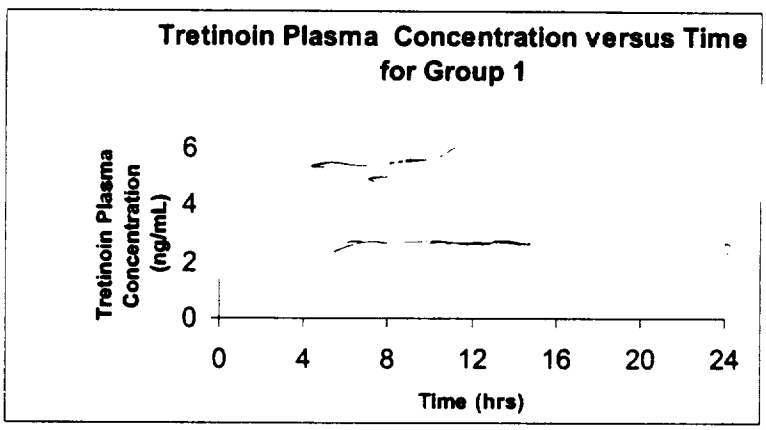
Tretinoin:

2. *Individual Plasma Concentrations:*

A summary of the results is presented below. An evaluation of the individual plasma concentrations is attached in Appendix 4 (pages 20-21).

Tretinoin:

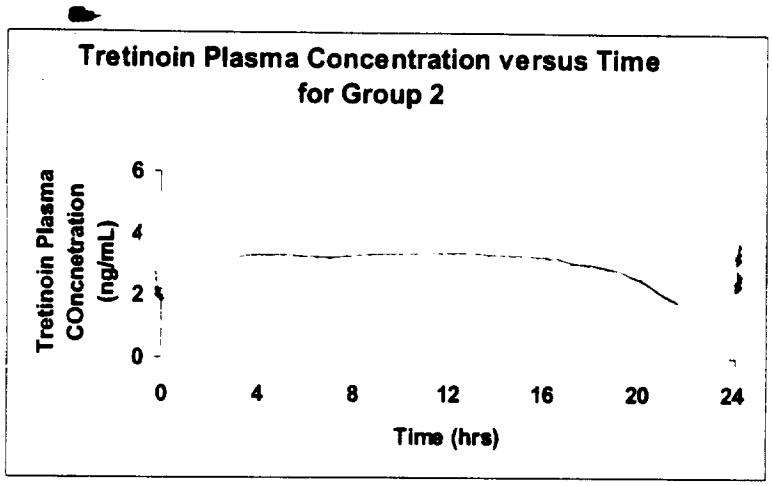
In group 1 26/45 (57.8%) of the subjects had quantifiable plasma tretinoin concentrations with a total number of 143/900 (15.9%). Reproduced in the graph below are the plasma tretinoin concentrations obtained for subjects in group 1 for all three sampling days (i.e. 1,7 and 14).



The plasma tretinoin concentrations ranged from [redacted] between the hours of 0-24 hrs post dose as shown in the graph above. The highest concentration was obtained at the [redacted] hour sampling time. The applicant stated that the endogenous blood concentration according to a publication by Thorne, EG, (British Journal of Dermatology (1992) 127, Supplement 41, 31-36) is 2-5 ng/mL. The plasma concentrations obtained in this study were all within the normal endogenous levels, indicating that the systemic exposure to TRI-LUMA™ cream does not result in an increase in the endogenous levels.

In group 2 8/14 (57.1%) of the subjects had quantifiable plasma tretinoin concentrations ranging from [redacted] between the hours of 0-24 hrs post dose. The total number of quantifiable plasma concentrations was 52/280 (18.6%), with the highest number of concentrations obtained on Day [redacted] Reproduced in the graph below

are the plasma- tretinoin concentrations obtained for subjects in group 2 for all three sampling days (i.e. 1,7 and 14).



The plasma tretinoin concentrations ranged from ——— ..... between the hours of 0-24 hrs post dose. The highest concentration was obtained at the ~ hour sampling time. These results demonstrated that there is very minimal difference in the systemic exposure to tretinoin following the application of the 1G and the 6G dose.

Fluocinolone acetonide:

No subject in both groups 1 and 2 had quantifiable plasma concentrations of fluocinolone at any of the sampling times evaluated. Although the group 1 assay method demonstrated that degradation was as high as 26%, however, the LOQ was 50 ng/mL and this is equivalent to 63 ng/mL in the absence of degradation. Therefore, if one factors in the degradation due to stability issues, the systemic exposure is still very minimal. The lack of quantifiable plasma concentrations from the group 2 study, with a higher dose and an assay method that had a lower LOQ (2ng/mL) also supports low systemic exposure.

Hydroquinone Concentrations:

Preliminary evaluation indicates that 8/45 (22%) of the subjects in group 1 had quantifiable plasma hydroquinone concentrations ranging from ? ——— between 0-24 hr post dose. In group 2 none of the subjects had measurable plasma concentrations of hydroquinone. Although the group 1 assay method demonstrated that degradation was as high as ~ —— ——— and the apparent highest concentration observed was ——— , and this is equivalent to ——— in the absence of degradation. Therefore, if one factors in the degradation due to stability issues, the systemic exposure is still very minimal. Also hydroquinone is currently included in the tentative final monograph for Over-the-Counter Skin Bleaching Drug Products [47 F.R. 39108-17 (9/3/82) 127]. One of the toxicity studies [by Lang, SN et. al. (Federation Proceedings, 9:74, 1950) to support safety was in 19 human volunteers, administered 300-500 mg (~1-2 times the dose applied topically) of hydroquinone orally for 3-5 months. The report concluded that none of the subjects showed any toxicity during the course of the experiment.

3. *Pharmacokinetic Parameters*

Although the applicant did include a table of the derived pharmacokinetic parameters, for hydroquinone and tretinoin (attached in the Appendix 4 page 22), the values shown for Cmax, Tmax and AUC do not correspond to the observed plasma concentrations or the derived individual pharmacokinetic parameters. By way of example, the Cmax obtained for Tretinoin on Day 1 was 1.34 (121) ng/mL, however, the observed Cmax from the raw plasma concentration time data for group 2 was 4.941 ng/mL for Day 1. Therefore, the applicant needs to re-calculate the pharmacokinetic parameters for labeling purposes.

C. Study Conclusions:

The data above indicate that the systemic exposure to tretinoin, fluocinolone acetonide and hydroxyquinone is minimal following daily application of TRI-LUMA ™ Cream (1G and 6G) for 8 weeks.

IV. Recommendations

The information submitted by the applicant addresses both deficiencies related to human pharmacokinetics and biopharmaceutics stated in the NA letter. The adrenal suppression study demonstrated that after 56 days treatment with TRI-LUMA™ cream only one patient had a slight reduction in adrenal response pre-cosyntropin stimulation. None of the subjects demonstrated adrenal suppression post-cosyntropin stimulation. However, after 28 days of treatment, there was one patient who was slightly suppressed both pre and post-stimulation. Following discussions with the medical reviewer (Dr. H. Ko) it was concurred that due to the slight contradictions seen with the data the applicant would need to incorporate this information in their label. The applicant included a precautionary statement in the label with regards to HPA axis suppression (see study abstract sheet in Appendix 1, page 12) and, the medical reviewer (Dr. H. Ko) is currently reviewing this.

The systemic absorption study following maximum exposure to TRI-LUMA™ cream indicates that systemic exposure to tretinoin, fluocinolone acetonide and hydroquinone following once daily application of 1G and 6 G for 8 weeks is minimal.

Based on the data submitted the applicant has addressed the deficiencies raised in the NA letter for TRI-LUMA ™ cream and, their application is acceptable from a clinical pharmacology and biopharmaceutics perspective. However, the applicant should adequately address the labeling comments and general comments below.

V. Labeling Comments:

The proposed draft label included in the proposed package insert under the heading "Pharmacokinetics" should be rewritten as follows:

Proposed Draft Label:

PHARMACOKINETICS:

VI. Comment to be conveyed to applicant as an additional information request:

The applicant needs to recalculate the derived pharmacokinetic parameters as the values given in the summary table and the proposed draft label are not consistent with the individual plasma concentrations and, individual derived pharmacokinetic parameters provided in the submission.

Abimbola O. Adebowale Ph.D.
Office of Clinical Pharmacology /Biopharmaceutics
Division of Pharmaceutical Evaluation III

RD/FT signed by Dennis Bashaw, Pharm.D.

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Appendix 1

Study Abstract Sheet: Protocol No. 33

Name of Investigational Product: TRILUMA Cream	**Name of Active Ingredient:** 0.01% fluocinolone acetonide + 4% hydroquinone + 0.05% tretinoin	**Indication:** Melasma of the face

Title of Study:
An adrenal suppression study of TRILUMA cream (0.01% fluocinolone acetonide + 4% hydroquinone + 0.05% tretinoin) in patients with melasma of the face

Principal Investigators; Study Centers:
Bruce Miller, M.D.
Oregon Medical Research
9495 S.W. Locust Street, Suite G
Portland, Oregon 97223-6683

Michael Jarratt, M.D.
DermResearch, Inc.
8140 North Mopac, Building 3
Austin, Texas 78759

Clinical Laboratory:	**Treatments Administered:**
Clinical Reference Laboratory 8433, Quivira Road, Lenexa, Kansas 66215	(Triluma Cream) by Hill Dermaceuticals, Inc. Lot K9900075

Objectives:
To evaluate the potential of TRILUMA Cream to suppress the HPA axis in patients with melasma

Mode of Administration:
Topical administration to the skin of the face as follows:
Approximately 30 minutes before bedtime and after washing the facial area with a mild cleanser (without scrubbing), patients were to apply a thin layer of the study medication to the entire face (~2 mg/cm^2 ≡360 mg maximum dosage for entire face). Special care was given to the hyperpigmented area making sure to cover the whole target area including the outside of its borders extending to the normally pigmented skin. Patients were to apply a mild cleanser daily and a moisturizer as needed. Patients were also to apply daily a sunscreen (further clarified in Amendment 1, 5 March 2001). Patients were supplied with the cleanser, moisturizer and sunscreen (with SPF 30 or greater with both UVB and UVA protection).

Dosing Regimen:	**Treatment Duration:**
Once-daily application (Dose was selected according to prior clinical and toxicological testing in published studies)	8 weeks

Study Population Demographics:	**Study Population Characteristics:**
Twenty-nine (29) patients (2 Male and 1 female) completed the study at 2 investigational sites (15 with Miller and 14 with Jarratt). The age of the patients ranged from 27 to 68 (Mean 49.2 ± 9.59). There were 23 (79.3%) Caucasians, 1 (3.4%) Asian and, 5 (17.2 %) Other.	*Skin Phototype*: Type I (4), Type II (13), Type III (11), Type IV (1) *Target Area Site*: Forehead only (1), Forehead and right/left cheeks (2), Right/left cheeks only (26) *Severity of Melasma*: Moderate (25 [86.21%]) and Severe (4 [13.79%]) *Normal functioning HPA axis defined by a serum cortisol level of at least 10 mcg/dL measured between 8-9 AM*

Design of Study:	**Study Schedule**
Phase II, controlled, open-label study	March 26 to June 7, 2001

Criteria for Evaluation:
Safety (primary):
Evaluation of HPA axis Function: Determination of serum cortisol levels pretreatment, Week 4 (day 28),

and Week 8 (day 56) just before and 60 minutes after injection of 0.25 Cosyntropin. All serum sampling for cortisol were conducted between 0734 and 0959, prior to receiving the applied dose of study medication for that study day. A normal response for study entry was defined as a pre-stimulation serum cortisol level between 10 µg/dL and 18 µg/dL. Cortisol levels below 10 µg/dL were considered unacceptable for study entry. A post-stimulation serum cortisol level greater than 18 µg/dL was considered a normal response. The laboratory reference range was 5-25 mcg/dL for cortoisol at 8 am. Patients with subnormal pre-stimulation serum cortisol levels (<10 µg/dL) and post-stimulation serum cortisol levels (<18 µg/dL) at the end of treatment were to be re-tested 7 days after the final dose and followed until normal pre- and post-stimulation levels were obtained.

Routine clinical laboratory tests (hematology, blood chemistry, urinalysis)

Adverse events

Others:

Global evaluation of melasma severity by investigator at each study visit

Global evaluation of improvement in melasma by investigator at each study visit

Global evaluation of improvement in melasma by patient at final visit

Weight of each tube was recorded before and after use, and was used to monitor patient compliance with the dosing regimen.

Statistical Methods:

Descriptive statistics and a paired t-test for comparison of means in Cosyntropin-stimulation test of HPA axis function for the different evaluation days. A p-value of ≤ 0.05 was statistically significant.

Summary Conclusions:

Individual Serum Cortisol Concentrations:

On day 1 (pretreatment) all 29 patients met the applicant's pre-specified criteria representative of a normal response (i.e. pre-stimulation serum cortisol levels (>10 µg/dL) and post-stimulation serum cortisol levels (>18 µg/dL). On day 28, five patients (#'s 2,3,4,11,26) had pre-stimulation serum cortisol levels < 10 mcg/dl ——— .) and one patient (#3) had post-stimulation serum cortisol levels < 18 mcg/dL. However, this patient also had subnormal ———) pre-stimulation serum cortisol levels on day 28. On day 56, one patient (# 4) had pre-stimulation serum cortisol levels < 10 mcg/dl ——— and all patients had post-stimulation serum cortisol levels >18 mcg/dL

There appeared to be a difference in the pre-stimulation response for Day1 versus Day 28. The clinical significance of this observation is unknown at this time since the findings were not consistent for the serum cortisol levels obtained after 56 days treatment. For a comparison of Day 1 versus Day 56, although there was one difference pre-stimulation, there was no difference post-stimulation.

Mean Serum Cortisol Concentrations

Comparison of pre- and post-stimulation (Cosyntropin) serum cortisol levels at Day 1, Day 28, and Day 56 showed a slight statistically significant difference in response Day 1 versus Day 28. The clinical relevance of this finding is unknown at this time. These results suggest that following 56 days of exposure to the corticosteroid (fluocinolone acetonide) in the formulation of TRILUMA Cream there was minimal evidence of adrenal suppression.

Adverse Events

The applicant stated that there were 3 patients with treatment-related adverse events involving the skin (desquamation, erythema, pruritus, discomfort). The events were mild to moderate and none of the patients discontinued prematurely. No serious adverse events were reported during the study.

Proposed Draft Label (Applicant stated this was obtained from class labeling for corticosteroids)

PRECAUTIONS

———

Cortisol levels (mcg/dL) **NDA 21-112 TRI-LUMA TM Cream** **Pretreatment (Day 1)**

Subject #	Age	Cosyntropin Pre-Stimulation	Cosyntropin (60 minutes) Post-Stimulation	Change from baseline	% Change from baseline
1	43				83.33
2	50				***164.35***
3	50				73.17
4	41				88.39
5	45				42.12
6	30				***126.72***
7	54				***92.65***
8	49				55.79
9	47				74.63
10	53				***108.05***
11	68				***111.11***
12	68				62.87
13	53				36.22
14	54				***146.46***
15	60				55.89
16	53				***98.65***
17	54				***135.76***
18	49				37.36
19	40				80.25
20	55				***119.44***
21	46				87.90
22	27				17.52
23	40				***179.28***
24	43				87.30
25	57				52.15
26	59				***157.41***
28	38				80.99
29	43				58.08
31	58				26.89
Average	**49.21**	**16.73**	**29.56**	**12.82**	**87.61**
Standard Deviation	**9.59**	**6.23**	**6.14**	**4.01**	**42.13**
CV%	**19.50**	**37.25**	**20.76**	**31.31**	**48.09**

* Bolded Italic number denotes subnormal values pre-stimulation (< 10 mcg/dL) or post stimulation (i.e. <18 mcg/dL) or approximate doubling of basal level at 60 minutes post stimulation.

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Cortisol levels (mcg/dL) NDA 21-112 Triluma Cream Day 28

Subject #	Age	Cosyntropin Pre-Stimulation	Cosyntropin (60 minutes) Post-Stimulation	Change from baseline	% Change from baseline
1	43				***114.39***
2	50				***233.33***
3	50				80.90
4	41				***141.76***
5	45				38.64
6	30				60.43
7	54				50.35
8	49				38.04
9	47				62.38
10	53				***100.00***
11	68				***190.32***
12	68				54.67
13	53				38.46
14	54				***124.56***
15	60				35.65
16	53				25.00
17	54				75.82
18	49				42.86
19	40				75.58
20	55				***169.64***
21	46				61.54
22	27				43.73
23	40				***113.67***
24	43				***115.07***
25	57				84.93
26	59				***156.25***
28	38				***249.15***
29	43				32.47
31	58				59.46
Average	**49.21**	**15.05**	**26.42**	**11.37**	**92.04**
Standard Deviation	**9.59**	**5.11**	**4.62**	**3.83**	**60.32**
CV%	**19.50**	**33.96**	**17.47**	**33.68**	**65.54**

* Bolded Italic number denotes subnormal values pre-stimulation (< 10 mcg/dL) or post stimulation (i.e. <18 mcg/dL) or approximate doubling of basal level at 60 minutes post stimulation.

NDA 21-112 Triluma Cream

Day 56

Cortisol levels (mcg/dL)

Subject #	Age	Cosyntropin Pre-Stimulation	Cosyntropin (60 minutes) Post-Stimulation	Change from baseline	% Change from baseline
1	43				66.45
2	50				***153.39***
3	50				8.06
4	41				***149.44***
5	45				59.21
6	30				***102.94***
7	54				81.34
8	49				72.38
9	47				46.77
10	53				88.46
11	68				***123.76***
12	68				89.38
14	54				***110.46***
15	60				68.94
16	53				72.57
17	54				80.75
18	49				76.76
19	40				60.80
20	55				***182.39***
21	46				***98.60***
22	27				49.00
23	40				53.37
24	43				48.39
25	57				85.81
26	59				***108.73***
28	38				80.00
29	43				87.18
31	58				21.88
Average	**49.07**	**17.65**	**30.68**	**13.03**	**83.13**
Standard Deviation	**9.74**	**6.28**	**7.23**	**4.57**	**37.89**
CV%	**19.85**	**35.60**	**23.55**	**35.05**	**45.58**

* Bolded Italic number denotes subnormal values pre-stimulation (< 10 mcg/dL) or post stimulation (i.e. <18 mcg/dL) or approximate doubling of basal level at 60 minutes post stimulation.

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Appendix 2

Study Abstract Sheet: Protocol No. 104479-70

Name of Investigational Product: ——— (TRILUMA) Cream	**Name of Active Ingredient:** 0.01% fluocinolone acetonide + 4% hydroquinone + 0.05% tretinoin	**Indication:** Melasma of the face

Title of Study:
An open-label study to determine maximum systemic exposure of ——— . cream

Principal Investigators; Study Centers:
Joseph Daddabbo, M.D.
Hill Top Research, Inc.
Main and Mill Streets
Miamiville, Ohio 45147

Analytical Methods Laboratory: ——— ———	**Treatments Administered:** ——— Cream (Triluma Cream) by Hill Dermaceuticals, Inc. (Lot 3 K980083, K990075, L990083) All were commercial formulations. Lot L990083 and K990075 used in clinical studies.
Design of Study: Phase I, open-label, non-randomized study with two groups applying two different doses (1G and 6 G)	**Comparator Product:** None

Study Schedule
Group I: January 17,2000 - March 20, 2000
Group II: September 10, 2000 – November 4, 2000

Objectives:
To determine the maximal systemic exposure, via percutaneous absorption under clinical use conditions for safety assessment, of ——— Cream following daily application for eight (8) weeks on healthy human volunteers.

Mode of Administration:
Prior to application the area will be cleansed with water. Subjects applied the material to either forearm or both forearms (depending on the group the subject belonged to) completely covering the entire area. Subjects were instructed to apply the test material in the morning at approximately the same time of day (± 2 hours). On days of visit to the test facility (1,2,4,7,8,14,15,21,35 and 56) group-II were instructed not to apply the cream at home as this will be applied at the research center.

Dosing Regimen: 1 G applied to the left or right arm between the wrist and elbow once-daily in the morning for Group I subjects 6 G applied simultaneously to cover each forearm between the wrist and elbow once-daily for Group II subjects	**Treatment Duration:** 8 weeks

Study Population Demographics: Forty-three (5M, 38F) subjects from Group I and thirteen subjects (2M, 11F) from Group II completed all phases of the study. Two subjects (016 & 037) discontinued study drug in group-I due to pruritus/burning. One subject (002) discontinued study drug in group-II due to pruritus. The age of the subjects ranged from 22 - 56 years old for group I and 26 – to 55 yeas old in group-II. There were 44 Caucasians and 1 African American in Group I and all Caucasians in Group II.	**Study Population Characteristics:** *Group I Skin Phototype*: Type II (13), Type III (28), Type IV (4) *Group II Skin*. Phototype: Type II (5), Type III (9),

Criteria for Evaluation:
Pharmacokinetic Sampling and Handling:
Approximately 30 mL of blood was collected as follows:
Day 1-prior to any treatment and at 2,4,6,8,12 and 24 hours post-treatment
Day 7-0, 2, 4, 6, 8, 12, and 24 hours post-treatment

Day 14- 0, 2, 4, 6, 8, 12 (± 30 minutes), and 24 hours (± 2 hours) post-treatment
Additionally samples will be obtained on Days 4, 21, 35 and 56 prior to treatment. Day 56 is a fasting (12 hours prior to visit) blood sample as part of the exit physical examination
Venous blood sample will be collected using an appropriate vacutainer collection system. Tubes were centrifuged at [redacted] and then plasma transferred to separate screw-cap tube and frozen at -80°C until analysis.

Analytical Methods: [redacted] was used for the quantitation of hydroquinone and fluocinolone acetonide with [redacted] respectively. Precision was ≤ 15% for hydroquinone and < 12% for fluocinolone acetonide. Both analytes (especially hydroquinone) were unstable in plasma after short term storage. This method was used for the quantitation of group I samples and for group II (only for hydroquinone) [redacted] method was used for the quantitation of tretinoin and fluocinolone acetonide with an [redacted] or both. Precision of the method was < 9% for fluocinolone acetonide and < 7% for tretinoin. This method was used for the quantitation of group II samples and group I (for tretinoin only).

Pharmacokinetic and Statistical Analysis:
Noncompartmental analysis was performed based on protocol sampling times and not actual sampling times Cmax and Tmax were observed values. AUClast was determined by the linear trapezoidal rule. Descriptive statistics were computed for the PK parameters by dosing group and analyte.

Summary Conclusions:
Hydroquinone Concentrations:
Preliminary evaluation indicates that 8/45 (22%) of the subjects in Group 1 had quantifiable plasma hydroquinone concentrations ranging from [redacted] between 0-24 hr post dose. The total number of quantifiable plasma concentrations were 15/900 (1.7%), with highest number of concentrations obtained on Day 14. In Group 2 none of the subjects had measurable plasma concentrations of hydroquinone. Trough hydroquinone plasma concentrations were [redacted] (Group 1) and [redacted] 2, indicating steady state was achieved by day 4.
Fluocinolone acetonide:
No subject in both groups 1 and 2 had quantifiable plasma concentrations of fluocinolone at any of the sampling times evaluated.
Tretinoin:
26/45 (57.8%) of the subjects in Group 1 had quantifiable plasma tretinoin concentrations ranging from [redacted] between the hours of 0-24 hrs post dose. The total number of quantifiable plasma concentrations was 143/900 (15.9%), with the highest number of concentrations obtained on Day 1 and, the highest concentration obtained at the 8 hour sampling time. 8/14 (57.1%) of the subjects in Group 2 had quantifiable plasma tretinoin concentrations ranging from [redacted] between the hours of 0-24 hrs post dose. The total number of quantifiable plasma concentrations was 52/280 (18.6%), with the highest number of concentrations obtained on Day 14 and, the highest concentration obtained at the 24 hour sampling time. The applicant stated that the endogenous blood concentration according to a publication by Thorne, 1992 is 2-5 ng/mL. The plasma concentrations obtained in this study were all within the endogenous levels, indicating that the systemic exposure to Triluma Cream does not result in an increase in the endogenous levels.
Conclusions: The systemic exposure to fluocinolone acetonide and tretinoin following daily application of Triluma Cream for 8 weeks is minimal. Although the results suggest that the systemic exposure to hydroquinone is also minimal, the stability issues of the assay method would not allow for a definitive conclusion at this time. The applicant included a table of the derived pharmacokinetic parameters, however the values obtained for Cmax and AUC did not correspond to the individual pharmacokinetic parameter values, the applicant needs to recalculate the values for these parameters.

Appendix 3

HPLC Assay Validation for Method Establishment (Pre-study): The assay method was validated by . Studies were initiated and completed as follows: January 17, to March 20, 2000 for group I and, September 10 to November 4, 2000 for group II. Human plasma Samples were analyzed from 01 August 2000 to 18 April 2001 (group I) and 15 February 2001 to 24th March 2001 (group II).

Compound		**Hydroquinone**	**Fluocinolone acetonide**
Internal Standard		**None**	**None**
Assay Method			
Matrix		Human Plasma	Human Plasma
Accuracy	***Within-Day***		
	Between-Day		
Precision (CV%)	***Within-Day***		
	Between-Day		
Standard curve range		25 – 1250 ng/mL (Non-linear > 0.9900) ≤ 10.7 % deviation from theoretical concentrations	50 – 2500 ng/mL (r = 0.0.9900) ≤ 1.8 % deviation from theoretical concentrations
Sensitivity (LOQ)			
Selectivity		No endogenous peaks at the retention times of hydroquinone that interferes with its quantitation.	No endogenous peaks at the retention times of fluocinolone acetonide that interferes with its quantitation.
Recovery Analyte	***(Mean % ±CV%)***	97.7 – 109 %	66.7 – 113 %
Stability		Storage @ Room temperature (~23°C) and for 4 hours prior to extraction < 30.5 % and < 13.8 % degradation was obtained respectively. After 3 freeze-thaw cycles < 13.6 % degradation was obtained. Long term storage @ - 80 °C for 12 days resulted in < 30.2% degradation All above with the lower concentration 50 ng/mL. The degradation for the higher (100 or 1000 ng/mL) concentration for all the above conditions was < 11.2%.	Storage @ Room temperature (~23 °C) and for 4 hours prior to extraction < 5.2 % and < 14.0 % degradation was obtained respectively. After 3 freeze-thaw cycles < 1.9 % degradation was obtained. Long term storage @ - 80 °C for 12 days resulted in < 26% degradation.

LC/MS/MS Assay Validation for Method Establishment (Pre-study): The assay method was validated by In Vitro Technologies, Inc., Baltimore, MD. Studies were initiated and completed as follows: January 17, to March 20, 2000 for group I and, September 10 to November 4, 2000 for group II. Human plasma Samples were analyzed from 01 August 2000 to 18 April, 2001 (group I) and 15 February 2001 to 24th March 2001 (group 2).

Compound		**Fluocinolone acetonide**	**Tretinoin**
Internal Standard		Testosterone	13-cis retinoic acid
Assay Method			
Matrix		Human Plasma	Human Plasma
Accuracy	***Within-Day***		
	Between-Day		
Precision (CV%)	***Within-Day***		
	Between-Day		
Standard curve range		2 – 100 ng/mL ($r > 0.990$) ≤ 6.7 % deviation from theoretical concentrations	2 – 100 ng/mL ($r > 0.990$) ≤ 3.4 % deviation from theoretical concentrations
Sensitivity (LOQ)			
Selectivity		No endogenous peaks at the retention times of fluocinolone acetonide that appeared to interfere with its quantitation.	No endogenous peaks at the retention times of tretinoin that interferes with its quantitation.
Recovery Analyte **Recovery Int. Std**	***Range*** ***Mean***	86.5 – 96 % 88.1 %	114 – 117 % 102 %
Stability		Storage @ Room temperature (~23 °C) for 24 hours resulted in < 0.9 % degradation. After 3 freeze-thaw cycles < 6.4 % degradation was obtained. Long term storage @ -80°C for 135 days resulted in < 12.7 % degradation.	Storage @ Room temperature (~23 °C) for 4 hours resulted in < 3.8 % degradation. After 3 freeze-thaw cycles < 7.3 % degradation was obtained. Long term storage @ - 80 °C for 8 days resulted in < 6.5 % degradation.

Conclusions: The method validation demonstrates that the HPLC analytical method used for quantitative measurement of hydroquinone and fluocinolone acetonide in human plasma are reproducible for the intended use, however, the stability results indicate that hydroquinone is unstable during both long term and short term storage and fluocinolone acetonide is unstable during long term storage. Also method is flawed in that no internal standard was used, therefore variability due to extraction methods was not controlled for in calculations. The applicant stated that the method could not be validated due to the stability issues with hydroquinone. The method validation for the [redacted] for the quantitation of fluocinolone acetonide and tretinoin was reproducible and accurate and is acceptable.

Appendix 4

Summary of Pharmacokinetic Results

Hydroquinone

Patient #	Hydroquinone Plasma Concentration (ng/mL)	Day	Time (hrs)
Group 1			
2		7	24
		14	0
		14	24
3		14	2
		14	4
4		7	12
5		14	2
15		1	8
		1	12
18		1	12
		14	8
19		1	6
26		14	4
Total =8/45 = 22%	= 15/900 = 1.7 %	1 = 4 4 = 1 7 = 2 14 = 7 35 = 1	0 = 3 2 = 2 4 = 2 6 = 1 8 = 2 12 = 3 24 = 2
Group II			

Fluocinolone Acetonide

Patient #	Fluocinolone Acetonide Plasma Concentration (ng/mL)	Day	Time (hrs)
Group 1			
Total = 0	0	0	0
Group II	**0**	**0**	**0**

Shaded text not post dose
The samples for all time points for patient # 16, Day 14 was lost during analysis.
The samples for Patient No's: 17 (day 1, 12hr), 21 (day 7, 8 hrs) 38 (day 7, 8hrs), 37 (day 56, 0) were not received.

Tretinoin

Summary Tables for Tretinoin Plasma Concentrations

Group 1								
Day	**1**	**7**	**14**	**4**	**21**	**35**	**56**	**Total**
No. of patients	**19**	**3**	**4**	**1A**	**14D**	**14D**	**14D**	**26/45**
No. of tretinoin plasma samples (ng/mL)	**52**	**40**	**31**	**4**	**7**	**4**	**5**	**143/900 =15.9%**
Plasma Concentration Range (ng/mL)								
Sampling Time (hrs)	**0**	**2**	**4**	**6**	**8**	**12**	**24**	
N	**38**	**14**	**20**	**20**	**14**	**16**	**21**	**143**
Plasma Concentration Range (ng/mL)								

Patient Nos. were 2,3,5,6,7,8,9,10,11,12,14,16,17,19,20,21,23,24,26,27,28,32,34,36,37,38

Group 2								
Day	**1**	**7**	**14**	**4**	**21**	**35**	**56**	**Total**
No. of patients	**5**	**3**	**7C**	**1E**	**7C**	**7C**	**7C**	**8/14**
No. of tretinoin plasma samples	**8**	**16**	**20**	**1**	**3**	**2**	**2**	**52/280 = 18.6%**
Plasma Concentration Range (ng/mL)								
Sampling Time (hrs)	**0**	**2**	**4**	**6**	**8**	**12**	**24**	
N	**12**	**5**	**8**	**6**	**7**	**5**	**9**	**52**
Plasma Concentration Range (ng/mL)								

Patient Nos. were 1,2,5,7,10,11,13,14

Sponsor's Calculations shown below does not correspond to the plasma concentrations above.

	Mean (% Coefficient of Variation)		
Day for Group 1 (1G dose)	**Cmax (ng/mL)**	**Tmax (h)**	**AUC last (ng.h/mL)**
1	**1.34 (121)**	**4.5 (171)**	**11.27 (195)**
7	**1.01 (153)**	**3.4 (206)**	**7.88 (304)**
14	**0.86 (165)**	**2.8 (238)**	**4.89(153)**
Day for Group 2 (6 G Dose)			
1	**1.27 (121)**	**2.6 (249)**	**3.48 (184)**
7	**1.42 (109)**	**6.9 (144)**	**11.80 (183)**
14	**1.43 (1260**	**5.6 (153)**	**12.63 (153)**

Summary of Non-Compartment Pharmacokinetic Parameters
Mean (% Coefficient of Variation) Values by Single Dose and Infusion

Group	Analyte	Day	C_{max} (ng/mL)	T_{max} (h)	AUC_{last} (ng.h/mL)
I*	Tretinoin	01	1.34 (121)	4.5 (171)	11.27 (195)
		07	1.01 (153)	3.4 (206)	7.88 (304)
1 gm dose		14	0.86 (165)	2.8 (238)	4.89 (153)
	Hydroquinone	01	2.85 (401)	0.6 (395)	8.47 (517)
		07	2.48 (470)	0.8 (495)	10.23 (537)
		14	5.75 (311)	0.4 (495)	15.78 (405)
	Fluocinolone Acetonide	01	0.00 (0)	0.0 (0)	0.00 (0)
		07	0.00 (0)	0.0 (0)	0.00 (0)
		14	0.00 (0)	0.0 (0)	0.00 (0)
II**	Tretinoin	01	1.27 (121)	2.6 (249)	3.48 (184)
		07	1.42 (109)	6.9 (144)	11.80 (183)
6 gm dose		14	1.43 (126)	5.6 (153)	12.63 (153)
	Hydroquinone	01	0.00 (0)	0.0 (0)	0.00 (0)
		07	0.00 (0)	0.0 (0)	0.00 (0)
		14	0.00 (0)	0.0 (0)	0.00 (0)
	Fluocinolone Acetonide	01	0.00 (0)	0.0 (0)	0.00 (0)
		07	0.00 (0)	0.3 (254)	0.00 (0)
		14	0.00 (0)	0.0 (0)	0.00 (0)

C_{max} Maximum or peak concentration over a 24-hour period
T_{max} Peak time over a 24-hour period
AUC_{last} Area under the whole blood/plasma concentration-time curve over a 24-hour period
* N = 45 subjects on study days 1 and 7; 44 subjects on study day 14
** N = 14 subjects

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Abi Adebowale
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Dennis Bashaw
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Clinical Pharmacology / Biopharmaceutics Review

NDA Number:	21-112 (Amendment)
Submission Date:	01/02/02 (Facsimile Transmission), 01/08/02
Product:	TRI-LUMA™ (fluocinolone acetonide 0.01%, hydroquinone 4.00%, and tretinoin 0.05%) Cream
Sponsor:	Hill Dermaceuticals, Inc., Sanford, Florida 32773-9311
Reviewer:	Abimbola Adebowale Ph.D.
Type of Submission:	A response to a biopharm information request to the original NDA amendment

Review of an NDA amendment to an Original NDA amendment under review

I. Background and Introduction

This amendment is in response to a biopharmaceutics information request faxed to the applicant on December 13th, 2001 for TRI-LUMA™ cream. The request conveyed to the applicant was as follows:

"The applicant needs to recalculate the derived pharmacokinetic parameters as the values given in the summary table and the proposed draft label are not consistent with the individual plasma concentrations and, individual derived pharmacokinetic parameters provided in the submission".

A review of the applicant's response is discussed in the next section.

II. Applicant's Response:

In the original amendment the applicant included all the subjects in the calculations of the derived pharmacokinetic parameters irrespective of whether they had quantifiable levels or not. In this submission the applicant re-calculated the derived pharmacokinetic parameters including only the subjects with quantifiable plasma concentrations of tretinoin and hydroquinone. Since all collected plasma samples of fluocinolone acetonide were below the limit of quantitation, there were no derived pharmacokinetic parameters to re-calculate. A copy of the applicant's response is attached in the Appendix. A summary of the re-calculated derived pharmacokinetic parameters are reproduced in the Tables below:

Hydroquinone

Table A:

	Mean (% Coefficient of Variation)		
Day for Group 1 (1G dose)	**Cmax (ng/mL)**	**Tmax (h)**	**AUC last (ng.h/mL)**
1 (N = 3)	42.71 (41.78)	8.7 (35.3)	127.00 (110.18)
7 (N = 2)	55.78 (8.83)	18.0 (47.1)	230.09 (77.13)
14 (N = 5)	50.59 (48.42)	3.2 (94.8)	138.87 (108.07)
Range of individual values for all three days combined	**25.55 – 86.52**	**0-24**	**26.3 – 364.0**

Tretinoin

Table B:

	Mean (% Coefficient of Variation)		
Day for Group 1 (1G dose)	**Cmax (ng/mL)**	**Tmax (h)**	**AUC last (ng.h/mL)**
1 (N = 20)	3.02(28.71)	10.2 (86.7)	25.36 (107.07)
7 (N = 15)	3.05 (31.94)	10.3 (87.4)	23.63 (96.72)
14 (N = 14)	2.92 (29.99)	9.4 (100.5)	16.53 (145.93)
Range for all three days combined	**2.01 - 5.336**	**0 - 24**	**0 - 85.4**
Day for Group 2 (6 G Dose)			
1 (N=6)	2.96 (36.41)	6.0 (150.6)	8.13 (96.46)
7 (N=6)	2.88 (26.26)	12.0 (82.3)	24.86 (113.98)
14 (N=6)	3.33 (27.86)	13.0 (65.8)	29.47 (65.47)
Range of individual values for all three days combined	**2.011 – 4.985**	**0 - 24**	**0 - 70.4**

The results in the above tables are consistent with the individual plasma concentrations and derived pharmacokinetic parameters included in the original amendment. The results in Table A above suggest that the derived pharmacokinetic parameters for hydroquinone and tretinoin appear to be similar for Days 1, 7 and 14 when one takes the variability associated with the values into consideration suggesting minimal accumulation. The derived pharmacokinetic parameters of tretinoin were also similar for groups I and II suggesting minimal difference in the systemic exposure following application of the 1G and 6G dose of TRI-LUMA cream.

III. Recommendations

The re-calculated derived pharmacokinetic parameters for tretinoin and hydroquinone included in this submission can be incorporated into the proposed draft label as appropriate since they are consistent with the individual plasma concentrations and, the individual derived pharmacokinetic parameters provided in the original amendment submission. Also the data in this submission still indicates that the systemic exposure to tretinoin, fluocinolone acetonide and hydroxyquinone is minimal following daily application of TRI-LUMA ™ Cream (1G and 6G) for 8 weeks. Therefore from the clinical pharmacology and biopharmaceutics this amendment is acceptable provided the applicant adequately addresses the labeling comments below.

IV. Labeling Comments

Proposed Draft Label:

The proposed draft label included in the proposed package is inserted below:

Revised proposed draft Label:

The proposed draft label should be revised as follows:

PHARMACOKINETICS:

it.

Abimbola O. Adebowale Ph.D.
Office of Clinical Pharmacology /Biopharmaceutics
Division of Pharmaceutical Evaluation III

RD/FT signed by Dennis Bashaw, Pharm.D.

APPENDIX

CREAM NDA

Biopharm Reviewer's Comment: The applicant needs to recalculate the derived pharmacokinetic parameters as the values given in the summary table and the proposed draft label are not consistent with the individual plasma concentrations and, individual derived pharmacokineitc parameters provided in the submission.

APPLICANT'S RESPONSE:

, method for the determination of plasma Tretinoin levels (Group I and II subjects) and Flucinolone acetonide (Group II subjects only) had a low limit of quantitation The HPLC method for the quantitation of Hydroquionone plasma levels and Fluocinolone acetonide plasma levels (Group I subjects only) had a low limit of quantitation f respectively.

In the original submission, Group I and II subjects had mean Tretinoin C_{max} values (Group I: 1.34 ng/mL on day 1, 1.01 ng/mL on day 7, and 0.86 ng/mL on day 14; Group II: 1.27 ng/mL on day 1, 1.42 ng/mL on day 7 and 1.43 ng/mL on day 14) that were substantially lower than the .. Such an outcome was caused by the fact that a large percentage of the subjects in both groups had plasma Tretinoin concentrations below the quantification limit (BQL) for all the sampling time points (Appendix 4 of the original submission).

With respect to Hydroquinone, the same behavior was observed.

With respect to Fluocinolone, the outcome was not controversial because all collected plasma samples showed either a zero concentration or a concentration below the quantification limit, indicating that the drug was not absorbed into the systemic circulation.

The applicant has re-calculate the mean C_{max}, T_{max}, and AUC_{last} on days 1, 7, and 14 for Tretinoin and Hydroquinone using a different method (i.e., only subjects with measuable drug levels were included in the calculation of mean values). The results of this re-calculation are presented in Table A (Tretinoin) and Table B (Hydroquinone).

For instance: Table A, of 45 Groups I subjects, only 20 had quantifiable plasma Tretinoin C_{max} values on day 1, resulting in a mean C_{max} was 3.02 ng/mL (i.e., using a demominator of 20 in place of 45 in the determination of the mean value). Table B, of 45 Group I subjects, only 3 had quantifiable plasma Hydroquinone C_{max} values on day 1 resulting in a mean C_{max} was 42.7 ng/mL.

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/s/

Abi Adebowale
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Dennis Bashaw
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Memorandum

Date: 01/17/02

From: Abi Adebowale, Ph.D.
Division of Pharmaceutical Evaluation III (HFD-880)

Through: E. Dennis Bashaw, Pharm. D.
Team Leader, Division of Pharmaceutical Evaluation III (HFD-880)

Subject: Response to specific issues raised in Form 483 that relate to bioanalytical validation data for Study M2000-019 included in NDA 21-112, TRI-LUMA Cream (fluocinolone acetonide 0.01%, hydroquinone 4.00% and tretinoin 0.05%)

To: Jonathan Wilkin, M.D., (Director) and
Victoria Lutwark (Project Manager)
Division of Dermatologic and Dental Drug products
(HFD-540)

Pursuant to an inspection, the Division of Scientific Investigations (FDA) issued a notice of inspection findings (FDA Form 483) on October 12th, 2001, to ——— ———. As part of the "483" specific issues related to the analytical validation of NDA 21-112 for TRI-LUMA cream (submitted on 7/20/01) were identified. For study report number M2000-019 (entitled "An open label safety study to determine maximum systemic exposure of ———) the issues were as follows:

"Accuracy of concentration results for study M2000-019 has not been assured in that

a. The HPLC methods for hydroquinone and fluocinolone acetonide in human plasma have not been validated.

b. Long-term storage stability for tretinoin in human plasma has only been validated for 8 days at -80°C. Subject samples were stored at -80°C for 5-12 months prior to analysis."

With regards to issue "a", the applicant submitted validated reports for the HPLC methods for fluocinolone acetonide and hydroquinone on November 1st, 2001 as an amendment to the pending application (NDA 21-112). The data have since been reviewed, found to be acceptable, and incorporated into the final review (dated 12/19/01).

In terms of issue "b" the inspector (Lynette P. Salisbury) also quoted in her report that ——— esponded that 12 month stability data should be available after August 2002". ——— ——— also reiterated this statement in their response (dated 11/25/01) to the October 12 Form FDA 483 page 3 under the subheading "Response to Observation # 2" as follows:

"Long-term stability for tretinoin in human plasma has been established for 8 days at –80° C. Stability samples have been continuously stored at –80° C for analysis after August

2002, which will provide stability data for at least 12 months of storage. These data will be appended to the validation report when they become available".

Therefore based on the above outlined communications between
and DSI-FDA, the Division of Pharmaceutical Evaluation III would like to make the following recommendations:

1. We acknowledge the observation in Form 483 that the HPLC method for hydroquinone and fluocinolone acetonide in human plasma was not validated however, since the sponsor has since submitted adequate validation reports to their NDA, this issue has been addressed.
2. In the final action letter to the applicant with regards to their NDA-21-112 submission, we suggest that the following comment be included:

   The Agency reminds the sponsor of their commitment to provide a final report on the 12 months storage stability of tretinoin in human plasma on or before August 2002.

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Abi Adebowale
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Dennis Bashaw
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