Pierre Fabre Dermatologie
Represented by the Institut de Recherche Pierre Fabre (IRPF)
45, Place Abel Gance
F-92100 Boulogne

CLINICAL STUDY REPORT

1. TITLE PAGE

HUMAN SKIN BLANCHING ASSAY COMPARING A NEW SHAMPOO CONTAINING BETAMETHASONE DIPROPIONATE AT 0.025% AND 0.050% TO THREE REFERENCE MARKETED FORMULATIONS IN HEALTHY SUBJECTS

Single-centre, investigator-blinded, active- and vehicle-controlled, intra-individual comparison

Investigational Product:	**0.025% and 0.050% betamethasone (dipropionate) shampoo (V0071 GM)**
EudraCT Number:	**2007-007315-93**
Protocol Number:	V00071 GM 1 01 1A
Phase of Development:	Phase Ia
Date of First Enrolment:	09 June 2008
Date of Last Completed:	11 July 2008
Principal Investigator:	Catherine QUEILLE-ROUSSEL, MD *CPCAD, Hôpital L'Archet 2* – 151 route de Saint Antoine de Ginestière - F06200 NICE Tel +33 (0)4 92 03 64 88
Sponsor Representative for Study Report:	Head of Therapeutic Area: Alain DELARUE, MD Tel +33 (0)5 34 50 61 88
Date of Report:	18 June 2013

Clinical trial performed in compliance with Good Clinical Practice

2. SYNOPSIS

Name of the Company: Pierre Fabre Dermatologie	**Individual Study Table Referring to part: of the Dossier:**	(FOR NATIONAL Authority Use only)
Name of finished product: **N/A**	**Volume:**	
Name of Active Ingredient: 0.025 and 0.050% betamethasone (dipropionate) shampoo (V0071 GM)	**Page:**	

Title of Study: Human skin blanching assay comparing a new shampoo containing betamethasone dipropionate at 0.025% and 0.050% to three reference marketed formulations in healthy subjects.

Principal Investigator: Dr Catherine QUEILLE-ROUSSEL

Study Centre:

Centre de Pharmacologie Clinique Appliquée à la Dermatologie (CPCAD)

Hôpital L'Archet 2 – 151 route de Saint Antoine de Ginestière –F06200 NICE

Publication (reference): Not written to date

Studied Period: 1 month *Date of first enrolment*: 9 June 2008 *Date of last completed*: 11 July 2008	**Phase of development:** Phase Ia

Primary Objective:

To determine the place of 2 dosages of a new betamethasone dipropionate shampoo (V0071 GM-0.025% and -0.050%) within the spectrum of topical corticosteroids formulation, by ranking their skin vasoconstriction potencies among those of established corticosteroid preparations.

Secondary Objective:

To assess the local and general safety of V0071 GM-0.025% and -0.050%.

Methods:

This study was conducted as a single centre, investigator blinded, active and vehicle-controlled, intra-individual comparison, involving 35 healthy subjects meeting specific inclusion/exclusion criteria.

The test products were randomly allocated to delineated 2.2 cm diameter sites on forearms. To avoid any overlap, each site was kept at least 2 cm apart from the other.

The study products were administered without occlusion as a short contact therapy for 5 minutes (min) for both test betamethasone shampoos and their corresponding vehicle and for 15 min for the Clobex® shampoo and both other reference lotions (Diprosone® and Localyn Glicole®).

Chromametric and visual assessments of vasoconstriction were performed[1] before product application (= baseline within 30 minutes before T0), then 4 hours (T_{4h}), 6 hours (T_{6h}), 8 hours (T_{8h}), 10 hours (T_{10h}), 12 hours (T_{12h}), 14 hours (T_{14h}) and 24 hours (T_{24h}) after T0 (product application time).

Chromametric measurements (using ChromaMeter Minolta) were performed as far as possible by the same trained person. Visual scoring was performed by two independent trained evaluators.

[1] **McKenzie AW and Stoughton RB.** *Method for Comparing Percutaneous Absorption of Steroids.* Arch. Dermatol. 1962;86:606-10.

Name of the Company: Pierre Fabre Dermatologie	**Individual Study Table Referring to part: of the Dossier:**	(FOR NATIONAL Authority Use only)
Name of finished product: **N/A**	**Volume:**	
Name of Active Ingredient: 0.025 and 0.050% betamethasone (dipropionate) shampoo (V0071 GM)	**Page:**	

Number of Subjects:

Overall, 35 subjects were randomised and (as planned) analysed.

Diagnosis and Main Criteria for Inclusion:

Female and male healthy subjects, aged between 18 and 50 years old with skin type II to IV on the Fitzpatrick scale[2] and demonstrating adequate vasoconstriction to topical corticosteroids Diprosone® lotion.

Test Product:

Name: N/A

Code: V0071 GM

Form: Shampoo

Concentration: Betamethasone dipropionate 0.025% and 0.050%

Dosage: 10 µL

Route of Administration: Topical application on the forearm under non-occlusive conditions

Frequency of Administration: Single application

Application Duration: 5 min, then rinsed with water and dried with a disposable tissue

Batch Numbers: #SB0647 for V0071 GM-0.025% and #SB0645 for V0071 GM-0.050% (expiry: October 2008 for both batches).

Matching Vehicle Control:

Name: N/A

Form: Shampoo

Concentration: 0%

Dosage: 10 µL

Route of Administration: Topical application on the forearm under non-occlusive conditions

Frequency of Administration: Single application

Application Duration: 5 min, then rinsed with water and dried with a disposable tissue

Batch Number: #SB0646 (expiry: September 2008)

Reference Product n°1:

Name: Clobex®

Form: Shampoo

Concentration: Clobetasol propionate 0.050% (very strong potency)

Dosage: 10 µL

Route of Administration: Topical application on the forearm under non-occlusive conditions

Frequency of Administration: Single application

Application Duration: 15 min, then rinsed with water and dried with a disposable tissue

Batch Number: 7114012 (expiry: November 2010)

[2] **Fitzpatrick TB.** *The Validity and Practicality of Sun-Reactive Skin Types I Through VI.* Arch Dermatol. 1988;124(6):869-71.

Name of the Company: Pierre Fabre Dermatologie	**Individual Study Table Referring to part: of the Dossier:**	(FOR NATIONAL Authority Use only)
Name of finished product: **N/A**	**Volume:**	
Name of Active Ingredient: 0.025 and 0.050% betamethasone (dipropionate) shampoo (V0071 GM)	**Page:**	

Reference Product n°2:

Name: Diprosone®

Form: Lotion

Concentration: Betamethasone dipropionate 0.050% (strong potency)

Dosage: 10 µL

Route of Administration: Topical application on the forearm under non-occlusive conditions

Frequency of Administration: Single application

Application Duration: 15 min, then wiped with a with a disposable tissue

Batch Number: 7009 (expiry: August 2009)

Reference Product n°3:

Name: Localyn Glicole®

Form: Lotion (solution)

Concentration: Fluocinolone acetonide 0.01% (moderate potency)

Dosage: 10 µL

Route of Administration: Topical application on the forearm under non-occlusive conditions

Frequency of Administration: Single application

Application Duration: 15 min, then wiped with a disposable tissue

Batch Number: M06G21 (expiry: May 2012)

Criteria for Evaluation:

Visual and colorimetric assessments of the vasoconstriction were performed before the application of products (within 30 min preceding T0) then 4 hours (T4h), 6 hours (T6h), 8 hours (T8h), 10 hours (T10h), 12 hours (T12h), 14 hours (T14h) and 24 hours (T24h) after the application of products (T0).

Main Pharmacodynamic Variable and Criterion:

The **main variable** was the **colorimetric parameter a* (reflectance)** measuring the **skin redness**. At each time, two successive series of measures were performed on each test site. For analyses, the mean of the two values was calculated. The results were expressed (per product/site) as the:

- Mean baseline-adjusted a* value (Δa*) per time,

- Area under the curve (calculated by the trapezoidal method) between T4h and T24h of Δa*: **Δa^*-AUC_{0-24h}** (**main criterion**).

Secondary Pharmacodynamic Criteria (1/2):

- Colorimetric parameter L* (luminance) measuring the **skin brightness**. At each time, two successive series of measures were performed on each test sites. For analyses, the mean of the two values was calculated. The results were expressed (per product) as the mean baseline-adjusted L* value (ΔL*) per time and by the ΔL^*-AUC_{0-24h}.

Name of the Company: Pierre Fabre Dermatologie	**Individual Study Table Referring to part: of the Dossier:**	(FOR NATIONAL Authority Use only)
Name of finished product: **N/A**	**Volume:**	
Name of Active Ingredient: 0.025 and 0.050% betamethasone (dipropionate) shampoo (V0071 GM)	**Page:**	

Secondary Pharmacodynamic Criteria (2/2):

- **Visual skin blanching response** (visual score ([VS]) according to a 5-point scale:
  - 0 = no change in skin colour,
  - 1 = slight (barely visible) blanching,
  - 2 = obvious blanching,
  - 3 = intense blanching,
  - 4 = blanching judged to be maximal.

Intermediate scores (of half unit) could have been used when needed.

The analysed variable was the mean between the two readers at each evaluation time.

The results were expressed by the mean values obtained at each assessment time, the total VS (TVS; sum of VSs), and the mean VS-$AUC_{0\text{-}24h}$. by product/site.

Safety Criteria:

Adverse events (AE) were assessed at each visit and reported on the AE pages of the CRF. Concomitant therapies taken during the study were documented.

Statistical Methods:

Demographics Analysis:

Quantitative descriptive statistics (n missing, mean, SD, SEM, median, minimum and maximum values) and qualitative (number and percentage of subjects in each class) were provided as appropriate.

Pharmacodynamics Analysis:

- The above-mentioned quantitative descriptive statistics were presented by product for: Δa^*-$AUC_{0\text{-}24h}$ (main criterion), Δa^* at T24h, VS-AUC, VS at T24h, ΔL^*-$AUC_{0\text{-}24h}$, ΔL^* at T24h,

- Mean values of each test variable (Δa^*, VS, and ΔL^*) were plotted per time and product.

- Prior to any statistical analysis, the normality of the test variable for each product/site was tested using the Shapiro-Wilk test.

=> In case of normality, an ANOVA-Latin square design was performed to test the treatment effect. In case of significant product effect ($p<0.05$), the products were compared using the Bonferroni adjusted p on the difference estimate.

=> In case of non-normality of the test variable for at least one product/site, the analysis of variance was replaced by a Kruskal-Wallis test and, in the case of significant treatment effect, the following partial paired comparisons were performed using the Wilcoxon test with Bonferroni adjustment:

- V0071 GM-0.025% with V0071 GM-0.050%,
- Then, V0071 GM-0.050% with each of the three reference products (very strong, strong and moderate potency) as well as with its vehicle and a non treated site.

- The analyses of the tests variables at T24h (Δa^* at T24h, VS at T24h, ΔL^* at T24h) were *post-hoc* analyses.

Safety Analysis:

Not applicable (no reported AEs or concomitant treatments)

Name of the Company: Pierre Fabre Dermatologie	**Individual Study Table Referring to part: of the Dossier:**	(FOR NATIONAL Authority Use only)
Name of finished product: **N/A**	**Volume:**	
Name of Active Ingredient: 0.025 and 0.050% betamethasone (dipropionate) shampoo (V0071 GM)	**Page:**	

Summary – Conclusions:

Subjects and demographics:

Sixty-five (65) subjects were screened and 35 were randomised. All the 35 randomised subjects completed the study course. No major deviation was observed in this study. All the subjects who entered the study fully satisfied the entry criteria. The age was 29.1 years on average and ranged between 20 and 44 years; 22 women (62.9%) were randomised; skin types II, III, and IV were represented at 40%, 54.3%, and 5.7%, respectively.

Pharmacodynamics (skin blanching response) results (1/2):

- Main variable: colorimetric parameter a*

A significant treatment effect was found on the skin blanching activity expressed by both the **Δa*-AUC$_{0\text{-}24h}$** (Kruskal-Wallis, p<0.001) and the **Δa* at T24h** (ANOVA-Latin square design, p<0.001).
The paired comparisons between V0071 GM-0.050% shampoo and each other product/site showed that the skin blanching activity of V0071 GM-0.050% expressed by the:

- **Mean Δa*-AUC$_{0\text{-}24h}$ (main criterion)**, was significantly weaker than that of the Diprosone® lotion (2.06 on V0071GM-0.050% vs. -11.87 on Diprosone®; Wilcoxon, p=0.0042). All other paired comparisons showed non-significant differences.
- **Mean Δa* at T24h** (*post-hoc*), was significantly weaker than those of the Diprosone® lotion (-0.36 on V0071GM-0.050% vs. -1.06 on Diprosone®; difference estimate: +0.7; ANOVA with Bonferroni adjustment p<0.0001) and the Clobex® shampoo (vs. -0.71 on Clobex®; difference estimate: +0.35; ANOVA with Bonferroni adjustment, p=0.0179). All other paired comparisons showed non significant differences.

- Secondary criteria:

o **Colorimetric parameter L***

A significant treatment effect was found on the skin blanching activity expressed by both the **ΔL*-AUC$_{0\text{-}24h}$** (ANOVA-Latin square design, p<0.001) and the **ΔL* at T24h** (Kruskal-Wallis, p<0.001).
The paired comparisons between V0071 GM-0.050% shampoo and each other product/site showed that the skin blanching activity of V0071GM-0.050% expressed by the:

- **Mean ΔL*-AUC$_{0\text{-}24h}$,** was significantly stronger than that of No product (-10.32 on V0071GM-0.050% vs. -16.91 with no product; mean difference estimate:+6.59; Wilcoxon with Bonferroni adjustment, p=0.0257), and significantly weaker than that of the Diprosone® lotion (vs. 2.63 on Diprosone®; difference estimate: -12.95; ANOVA with Bonferroni adjustment (p<0.0001). All other paired comparisons showed non significant differences.
- **Mean ΔL* at T24h** (*post-hoc*), was significantly weaker than that of the Diprosone® lotion (-0.27 on V0071GM-0.050% vs. 0.51 on Diprosone®; Wilcoxon with Bonferroni adjustment, p=0.0223). All other paired comparisons showed non significant differences.

Name of the Company: Pierre Fabre Dermatologie	**Individual Study Table Referring to part: of the Dossier:**	(FOR NATIONAL Authority Use only)
Name of finished product: **N/A**	**Volume:**	
Name of Active Ingredient: 0.025 and 0.050% betamethasone (dipropionate) shampoo (V0071 GM)	**Page:**	

Pharmacodynamics (skin blanching response) results (2/2):

- Secondary criteria (2/2):

- **Visual score (VS)**

A significant treatment effect was found on the skin blanching activity expressed by both the **VS-AUC$_{0\text{-}24h}$** and the **VS at T24h** (Kruskal-Wallis, $p<0.001$ in both cases).
The paired comparisons between V0071 GM-0.050% shampoo and each other product/site showed that the skin blanching activity of V0071GM-0.050% expressed by the:

- **VS-AUC$_{0\text{-}24h}$**, was significantly stronger than those of the Vehicle (13.16 on V0071GM-0.050% vs. 6.90 on the Vehicle; Wilcoxon, $p=0.0107$) and No product (vs. 5.63 with no product; Wilcoxon with Bonferroni adjustment, $p=0.0004$), and significantly weaker than that of the Diprosone® lotion (vs. 29.06 on Diprosone®; Wilcoxon with Bonferroni adjustment, $p<0.0001$). All other paired comparisons showed non significant differences;
- **TVS** showed similar results as those obtained with the VS-AUC$_{0\text{-}24h}$;
- **VS at T24h** *(post-hoc)*, was significantly stronger than those of the Vehicle (0.74 on V0071GM vs. 0.32 on Vehicle; Wilcoxon with Bonferroni adjustment, $p=0.0016$) and No product (vs. 0.33 with no product; Wilcoxon with Bonferroni adjustment, $p=0.0019$), and significantly weaker than that of the Diprosone® lotion (vs. 1.61 on Diprosone® lotion; Wilcoxon with Bonferroni adjustment, $p<0.0001$). All other paired comparisons showed non significant differences.

Safety results:

No adverse event was reported during the study course.

Conclusion:

The blanching activity of V0071 GM-0.050% was shown to be:

- Significantly stronger than that of no product control (all criteria derived from both ΔL* and the visual score) and its vehicle (all criteria derived from the visual score);
- Significantly weaker than that of the very strongly potent Clobex® shampoo (Δa* at T24h);
- Non different from that of V0071 GM-0.025% shampoo (all parameters).

Corticosteroid lotions did not prove to be appropriate reference products to rank corticosteroid shampoo formulations' activity.

The study products were all well tolerated, with no adverse events reported during the study course.