**PROTOCOL SYNOPSIS**

<table>
<thead>
<tr>
<th>I. PROTOCOL TITLE:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation of the efficacy and safety of indigo naturalis oil extract and calcipotriol solution in patients with nail psoriasis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. OBJECTIVES:</th>
</tr>
</thead>
<tbody>
<tr>
<td>✷ To compare the efficacy and safety of indigo naturalis extract in oil (Lindioil) to calcipotriol solution in patients with nail psoriasis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>III. TEST DRUG:</th>
</tr>
</thead>
</table>
| 1. Name: Experimental: Indigo naturalis extract in oil (Lindioil)  
Comparator: Calcipotriol solution |
| 2. Dosage form: liquid form |
| 3. Dose(s): Experimental: Indirubin 200 μg/ml  
Comparator: Calcipotriol 50 μg/ml |
| 4. Dosing schedule: apply one to two drops (0.05 ml per drop) of either Lindioil or calcipotriol solution twice daily onto the lateral and proximal nail folds plus the hyponychium of affected nails |
| 5. Mechanism of action (if known): |
| ✷ Indigo naturalis regulates hyperproliferation and abnormal differentiation in epithelial keratinocytes. |
| ✷ Indigo naturalis suppresses tumor necrosis factor-α-induced vascular cell adhesion molecules-1 expression in vascular endothelial cells. |
| ✷ Indirubin exhibits anti-inflammatory effects via inhibition of interferon-γ, interleukin-6 production as well as TNBC-induced delayed-type hypersensitivity. |
| ✷ Indigo naturalis inhibits \( \mathrm{O}_2^- \) generation and elastase release in FMLP-induced human neutrophils. |
| ✷ Indirubin inhibits DNA synthesis in various cell lines, such as leukemia cells and breast carcinoma cells, and selectively inhibit CDKs and other kinases, as well as block cancer cell proliferation in the late G1 and G2/M phases of the cell cycle. |
| ✷ Indirubin enhances tumor necrosis factor-induced apoptosis through modulation of the nuclear factor-κB signaling pathway. |
| ✷ Indirubin reduces both the expression and production of RANTES in influenza virus-infected human bronchial epithelial cells. |
| 6. Pharmacological category: |
| ✷ Anti-inflammatory |
| ✷ Anti-tumor |
| ✷ Anti-bacteria |
IV. DEVELOPMENT PHASE:

Phase □ I □ II □ III □ IV ■ Others: Research

V. STUDY DESIGN:

1. ■ Control: □ Placebo
   ■ Active
   □ Others
   □ Uncontrolled:


3. Randomized: ■ Yes □ No

4. Parallel □ Cross-over ■ Others

5. Duration of study: __24 weeks____

6. Titration: □ Forced □ Optional ■ None

7. □ Multi-national ■ Multi-center (Taiwan) □ Single-center (Taiwan)

8. Number of subjects: ______ 33 subjects ______

9. Is there any of the followings included DSMB, Data Safety Monitoring Board:
   □ Yes ■ No

VI. SAMPLE SIZE ESTIMATION:

The sample size of 33 was calculated based on the following: (1) mixed effect model with two within-factors (treatments and time), (2) the change in shNAPSI score or mtNAPSI score between two treatments, (3) intra-class correlation of 0.9, (4) the significance level is 0.05, and (5) the statistical power is 0.9.

VII. ENDPOINTS:

Efficacy Endpoints

Primary endpoint:
Evaluate the efficacy by comparing the mean percentage change in single hand Nail Psoriasis Severity Index (shNAPSI) scores between baseline (week 0) and week 24

Secondary endpoints:

1. Measure the mean percentage change in modified target NAPSI (mtNAPSI) scores for the single most severely affected nail from baseline (week 0) to week 24

2. Evaluate the subject’s global assessment (SGA) and physician’s global assessment (PGA) at week 24

Safety Endpoints

1. Vital signs
2. Physical examinations
3. Adverse events

VIII. SELECTION CRITERIA:

Inclusion Criteria
1. Male or female, 20 to 65 years old.
2. Subjects have symmetrically comparable psoriatic nails on both hands.
3. Subjects are in good general health, without abnormal liver, renal function, or significant abnormalities in hematology.
4. Female patients of child-bearing age who have agreed to continue using birth control measures as approved by the investigator and agree not to lactate for the duration of the study.

Exclusion Criteria
1. Concomitant topical treatment (corticosteroids), phototherapy, or any systemic treatment (retinoids, cyclosporine, methotrexate, systemic corticosteroids and biological agents) that could affect nail psoriasis.
2. Patients with severe hepatic or renal disorders.
3. Lactating, pregnant or planning pregnancy.
4. Unwillingness to comply with study protocol.
5. A history of sensitivity to indigo naturalis.

Withdrawal Criteria
1. Adverse events (AEs) that develop possibly related to the treatment, nail psoriasis becomes worse, or withdraw their consent during the study.
2. Poor compliance.

IX. STUDY PROCEDURES:

This is a randomized, observer-blind, active-controlled, intra-subject trial and will be conducted in 3 branches of Chang-Gung Medical Foundation (Keelung, Linkou and Taipei). The study aims to compare the efficacy and safety of indigo naturalis extract in oil (Lindioil) to topical calcipotriol solution in treating nail psoriasis.

We plan to enroll a total of 33 eligible subjects (to achieve 26 evaluable) with symmetrically comparable nail psoriasis on each hand. The subjects who meet the eligibility criteria will be instructed to apply Lindioil to the fingernails of one hand and calcipotriol solution to the other hand twice daily for 24 weeks. The blocked randomization method was used with 1:1 ratio for both treatments. During the treatment period, any anti-psoriatic nail treatments other than study drugs are
prohibited. Treatment could be stopped at any time upon completed clearance, or if there is any significant local or systematic AE possibly related to treatment.

The safety and efficacy of the test drugs will be assessed by the following methods: (1) The investigator will assess the single hand Nail Psoriasis Severity Index (shNAPSI, 0 ~ 40) and modified target NAPSI (mtNAPSI, 0 ~ 96). (2) Physician and subjects will evaluate the overall improvement using physician’s global assessment (PGA, 0 ~5) and subject global assessments (SGA, 0 ~ 5); (3) Safety will be monitored by measuring vital signs, physical examinations as well as any occurrence of adverse events.

X. CONCOMITANT TREATMENT:

Prohibited

Investigators should minimize the concomitant medications for subjects during the trial in order to prevent possible interference with study endpoints. Any anti-psoriatic nail medications/therapies are NOT ALLOWED for subjects during the study.

Permitted

If concomitant medication is deemed necessary, investigators should maintain a stable dosage and type of therapy during the study in order to minimize possible interferences for the study endpoint assessments. Information regarding every concomitant medication, once used during study period, must be recorded on the Case Report Form, including the therapy type, dose regime, date of administration, etc.

XI. STATISTICAL ANALYSIS:

1. Primary hypothesis: ■ Superiority □ Non-inferiority □ Equivalence □ Other
2. Sample size: Enrolled: ___33___ Evaluable: ___26___
3. Efficacy population: ■ ITT □ PP □ Other
4. Safety population: □ ITT □ PP ■ Other: Safety
5. Statistical method(s) for efficacy/safety evaluations:

Continuous variables such as subject age and duration of psoriasis are expressed as mean ± SD or as a percentage. Categorical variables, such as gender, are expressed as frequency or percentage. The mixed-effect model with two within-factors (treatments and time) was used to account for time dependency of the repeated measurements and differences between the two hands. Pearson correlation coefficient was used to test the correlation between SGA and PGA. The comparison of the efficacy of the different treatments was conducted by a paired-T test. The overall significance
The significance level of this study was 0.05; however, the Bonferroni method reduced the significance level. This was accomplished by dividing the numbers of multiple comparisons when comparing shNASPI (or mtNAPSI) between the two hands over time. In other words, when comparing shNASPI (or mtNAPSI) between the two hands over time, the significance level was .007 (= .05/7 visit). SAS 9.3 was used to conduct the statistical analysis.

6. Planned interim analysis: □ Yes ■ No