Final Study Report

Study Title: Vitiligo en het Koebnerfenomeen (Vitiligo inductie- en therapie model : klinische en immunologische analyse)

EudraCT number: 2009-017425-19

Study protocol code: AG0/2009/012

ClinicalTrial.gov identifier: NCT01082393

Sponsor: UZ Ghent

National Coordinator/ Coordinating Investigator: Prof. dr. N. Van Geel

Date of report: 22 Nov 2019

Name and signature Sponsor: Prof. N. van Geel

Date signature Sponsor: .....

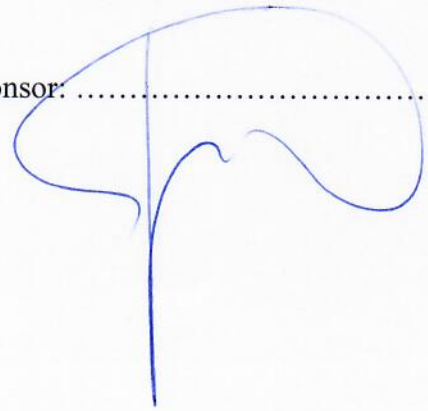

Table of content

1. Introduction

With this study we hope to gain more insight into the underlying cause of the skin disease vitiligo (= pigment disorder where white spots occur at the level of the skin).

The research consists of 3 research sections. In **part 1**, cream therapies are evaluated with an active ingredient (medicine), while in **parts 2 and 3** we focus primarily on the "control groups". **Part 2** is the first "control group". This will consist of patients with vitiligo. In this group, no additional cream therapy will be applied or (and) only cream therapy will be used without an active ingredient (moisturizing cream). In **part 3** we focus on the second "control group". This will consist of patients without vitiligo, for whom we will only apply a limited part of the intervention.

For parts 1 and 2 of this study, only patients with a very extensive form of vitiligo (> 50% BSA) or patients who are under a depigmenting (decolouring) treatment at the time of the study can be included. In these patients, a further discoloration of the skin is aimed for, with the aim of obtaining an even (discolored) skin, by means of the Koebner phenomenon. The Koebner phenomenon with vitiligo can be any stimulating factor that induces further depigmentation such as cryotherapy (freezing), laser therapy, superficial skin abrasion. An attempt will be made in this study to provoke this koebner phenomenon, but moreover an attempt will be made to counter this process via topical therapies. The therapeutic value and the mechanism of action of different topical products can thus be mapped and, moreover, the different forms of treatment can be compared with each other. This research will also provide more insight into the "koebnerization process" and thus contribute to the unraveling of the pathogenetic mechanism of vitiligo. In addition, information will be obtained about the efficiency of certain depigmenting treatments in vitiligo, including in function of frequency of application and in function of body localization.

For part 3, patients without vitiligo will be included, for whom a small removal to the skin is planned or not. The skin reaction that we will induce in this patient group will be compared with that of patients with vitiligo.

2. Objectives of the study

2.1 Primary objectives

Analysis of clinical and immunological processes that arise during the induction phase of vitiligo and the influence of topical therapies on this process.

3. Investigational Medicinal Product

Samenstelling en dosering

Products that can be used in PART 1 of the study

Elocom lipophilic cream

Composition:

Mometasone furoate 1mg, in White Vaseline, White beeswax, Propylene glycol stearate, Stearyl alcohol and Ceteareth 20, Hexylene glyco, Titanium dioxide, Diluted phosphoric acid, Rectified corn starch, Purified water.

Dosage: 3 cutaneous applications in total (day 1, day 3, day 6)

Protopic ointment

Composition:

tacrolimus monohydrate 0.1% in ointment base (ointment base = white paraffin, liquid paraffin, propylene carbonate, white beeswax, hard paraffin)

Dosage: 3 cutaneous applications in total (day 1, day 3, day 6)

Elidel cream
Composition:
pimecrolimus 1%. In cream base (cream base = triglycerides, oleyl alcohol, propylene glycol, stearyl alcohol, cetyl alcohol, mono- and diglycerides, sodium cetostearyl sulfate, benzyl alcohol, citric acid (anhydrous), sodium hydroxide and purified water).
Dosage: 3 cutaneous applications in total (day 1, day 3, day 6)

Product used in PART 1 and PART 2 of the study

Moisturizing cream (Cold cream)
Composition:
Arachidis oleum, Cera alba, Monoleinum, Rosa aetheroleum, Aqua purificato
Dosage:
Part 1: 3 cutaneous applications in total (day 1, day 3, day 6)
Part 2:1x per day cutaneous application to start 2 weeks prior to day 1 up to at least 1 month and up to 6 months after day 1.

No additional creams are used in PART 3 of the study

Marketing Authorization Holder - Distributor

1. Elocom cream (Mometason furoate, 1 mg / g): NV Schering-Plow Labo, Industriepark 30, 2220 Heist-op-den-Berg
2. Protopic 0.1% ointment (tacrolimus, 1 mg / g): Astellas Pharma B.V. Branch, Erasmus Park, Square Marie Curie 50, 1070 Brussels
3. Elidel 1% cream (pimecrolimus 10 mg / 1 g): Novartis Pharma, NV.Medialaan 40, B-1800 Vilvoorde
4. Cold Cream (Cold cream Lipocrème): Fagron NV / SA, textile street 20, B-8790 Waregem

Distributor

Hospital pharmacy UZ Ghent

Packaging

The commercially available packaging will be used

Administration route

Topical application

Labeling

Original label will not be changed.
Application days + frequency will be noted on the package

4. Study Protocol Summary

The research consists of 3 research sections.

In **part 1**, cream therapies are evaluated with an active ingredient (medicine), while in **parts 2 and 3** we focus primarily on the "control groups". **Part 2** is the first "control group". This will consist of patients with vitiligo. In this group no additional cream therapy will be applied or (and) only a cream therapy will be used without an active ingredient (moisturizing cream). In **part 3** we focus on the second "control group". This will consist of patients without vitiligo, for whom we will only apply a limited part of the intervention.

The following applies to **part 1**: Randomized intra-individually controlled double blind (patient blind and blind assessor of photographic material).

For **part 2**, the following applies: Randomized intra-individually controlled single blind (blind assessor of photographic material) study.

For **Part 3**, the following applies: Randomized intra-individually controlled single blind (blind assessor of photographic material) study.

Inclusion criteria

PART 1 and 2

- Extensive form of vitiligo (> 50% BSA) or vitiligo patients who are under depigmenting / decolouring treatment
- Patients who request depigmenting therapy
- 18 years or older
- Not pregnant

PART 3

- Patients without vitiligo who come to the dermatology department for consultation
- 18 years or older
- Not pregnant

4.1 Exclusion criteria

- Vitiligo patients who do not request depigmenting therapy
- Children up to and including 17 years old
- Pregnancy

4.2 Primary endpoint

- Analysis of the photos:
  1. by a "blind" person
  2. using. a semi-automatic digital image processing system (ImageJ software)
- Analysis of histology, immunohistochemistry: using. confocal microscope
- Analysis of immunotyping: inter alia by means of FACs analysis lesionally isolated T cells
- T cell receptor analysis lesional lymphocytes

4.3 Procedures

PART 1

Day 1

During the first visit (day 1), a maximum of 15 different test zones (approximately 2.5 x 2.5 cm) will be photographed and treated in 3 different ways with the aim of inducing skin discoloration. The number of test zones will depend on the number of suitable test areas on the body. After the treatment, different creams will be applied, which will then be covered with a dressing. This must remain on site until the next visit. If the patient agrees, a small skin biopsy will also be taken on this day. The latter takes place under local anesthesia of the skin. The biopsy site will be sutured afterwards.

Day 3 (± 1 day)

During the visit on day 3, all test zones will be viewed and photographed. A new cream will be applied again under a new dressing. This must remain on site until the next visit. If the patient agrees with this, 2 to 6 small skin biopsies will also be taken on this day. The biopsy site will be sutured afterwards.

Day 6 (± 1 day)

During the visit on day 6, all test zones will be viewed and photographed. A new cream will be applied again under a new dressing. This must remain on site until the next visit.

Day 10 (± 2 days)

During the visit on day 10, all test zones will again be viewed and photographed. From then on no cream or bandage is needed anymore. If the patient agrees with this, 2 to 6 small skin biopsies will also be taken on this day. The biopsy sites will be sutured afterwards.

Day 30 (± 7 days)
During the visit on day 30, all test zones will be viewed and photographed again. If the patient agrees with this, 2 to 6 small skin biopsies will also be taken on this day. The biopsy sites will be sutured afterwards.

Day 60 (± 7 days)
During the visit on day 60, all test zones will again be viewed and photographed. If the patient agrees with this, 2 to 6 small skin biopsies will also be taken on this day. The biopsy sites will be sutured afterwards.

Day ± 180
When you visit this day, all test zones will be viewed and photographed again.

PART 2
Day 0
Two weeks prior to the start of the (first) vitiligo provocation test, it may be necessary to apply a moisturizing cream (for example cold cream) once a day to a clearly agreed skin area.

Day 1
At the first visit (day 1), a minimum of 2 to a maximum of 12 different test zones (approximately 2.5 x 2.5 cm) will be photographed and treated in 1 to a maximum of 3 different ways with the aim of inducing skin discoloration. The same test or part of the same tests may be performed at different body locations. The number of test zones will depend on the number of suitable test areas on the body and will also be selected in consultation with the patient. After the vitiligo provocation test, the patient may be asked to apply the moisturizing cream further once a few selected test zones (for a minimum of 1 to a maximum of 6 months). If the patient agrees, a small skin biopsy may be taken on this day. The latter takes place under local anesthesia of the skin.

Day 10-14 (± 2 days):
When visiting this day, all test zones will be viewed and photographed. If the patient agrees, a small skin biopsy may be taken on this day.

Day 21 (± 7 days)
During the visit on this day, all test zones will be viewed and photographed. The vitiligo challenge test may be repeated again on 1 or a few existing test zones. If the patient agrees, a small skin biopsy will also be taken on this day.

Day 42 (± 14 days)
During the visit on this day, all test zones will be viewed and photographed. The vitiligo challenge test may be repeated again on 1 or a few existing test zones.

Day 63 (± 21 days)
During the visit on this day, all test zones will be viewed and photographed again. The vitiligo challenge test may be repeated again on 1 or a few existing test zones.

Day 180 (± 30 days)
During the visit on this day, all test zones will be viewed and photographed again.

PART 3
Day 1
At the first visit, a minimum of 1 to a maximum of 3 different test zones (a maximum of 2.5 by 2.5 cm) will be photographed and treated in 1 to a maximum of 3 different ways with the aim of inducing a skin reaction (day 1). The number of test zones will depend on the number of suitable test areas on the body and will be determined in consultation with the patient.

± Day 10-14:
During the visit on this day, the test zone (s) will be viewed and photographed. On this day a small skin biopsy will be taken or the removal will take place that was already planned outside this study. This biopsy / removal takes place under local anesthesia of the skin. This means that you will get a small puncture at that location,

which will make the skin temporarily numb. A very small piece of skin will then be removed. The biopsy / removal site will be sutured afterwards. Once the local anesthetic has been worked out, this will hardly be a problem (at most a slight tight feeling which disappears after 1 to 2 days).

The expected total duration of the study will be a maximum of 180 days for the patient in parts 1 and 2 and a maximum of 14 days for part 3.

The expected total duration of the entire study is approximately 5 years.

4.4 Randomisation and blinding

Blinding of the assessor: the clinical evaluation (percentage depigmentation versus pigmentation) will take place based on. clinical photos. The assessment of histological material will be based on Skin sections.
The assessor is a person who will not receive information about the intervention.
Only after completion of the assessment will the randomization code be broken.

The creams will be applied ad randomly to one of the selected test zones.

Randomization will take place using a draw system.

5. Study analysis

This is an intra-individually controlled study.

Only 1 patient can already provide us with a lot of information.

The minimum is therefore 1, but for confirmation of our results we aim for 20 to 45 patients (PART 1: 15 people; PART 2: 20 people; PART 3: 10 people).

6. Independent Ethics Committee and Competent Authority

The following documents have been approved by the EC and the CA:

OVERVIEW APPROVED DOCUMENTS		
Initial submission : - Protocol, version 1.0, 20NOV2009 - ICF, 18DEC2009 -	**Approval EC:** 24DEC2009	**Approval FAGG:** 10DEC2009
Amendment 1: - Protocol, version 2.0, 18JAN2010 -	**Approval EC:** 04FEB2010	**Approval FAGG :** 12FEB2010
Amendment 2: - Protocol, version 3.0, 26MAR2010 - ICF, 26MAR2010 -	**Approval EC:** 02APR2010	**Approval FAGG :** N.A.
Amendment 3: - Protocol, version 4.0, 02AUG2012 - ICF, 02AUG2012	**Approval EC:** 18SEP2012	**Approval FAGG :** 26SEP2012

Amendment 4:	Approval EC:	Approval FAGG :
- Addition test zones skin in protocol, version 4.0, 02AUG2012 - ICF, 13MAY2014	05JUN2014	N.A.

7. Results

7.1 Subject enrollment and demographics

14 patients were screened for the study, of which 12 were randomised and completed the study. First patient first visit occurred on 16-Feb-2010, last patient last visit on 15-Sep-2015. 2 of the screened patients have refused further participation and have been considered to be drop-outs.

45 patients were planned to be included.

Based on the number of included patients sufficient data collection was available to complete the study.

7.2 Study specific results

We used our "in vivo vitiligo induction and therapy model" in 3 patients and matched controls.

With this model we were able to investigate the clinical, histopathological and immunological process of vitiligo induction [experimental Koebner phenomenon (KP)]. We focused mainly on:

1) Reproducibility and value of this model.
2) Evaluation of 3 cream treatments and their effect on the immune balance.
3) Lesional T cell infiltrate before and after Koebner (vitiligo) induction.

Comparative immunohistochemcal analyses and flow cytometric analyses were performed (17 skin biopsies). Lesional and non-lesional melanocyte specific cytotoxic T lymphocyte responses against melanocyte differentiation antigens (Mart-1, gp100 and tyrosinase) were evaluated by flow cytometry staining using HLA-A2 specific tetramers on T cells isolated from fresh tissue biopsies. This was performed in collaboration with Dr. R. Luiten, Academic Medical Center, Amsterdam.

These resulted in the following publication:
Reference: van Geel N, et al. In vivo vitiligo induction and therapy model: double-blind, randomized clinical trial. Pigment Cell Melanoma Res. 2012 Jan;25(1):57-65.

We could demonstrate that our *in vivo* vitiligo induction model is very informative to investigate vitiligo induction and to determine the efficacy of topical treatments in vitiligo. The amount of

CD4+ andCD8+ T cells increased markedly after koebnerization and could be partly prevented by topical anti-inflammatory therapy (mometasone furoate, pimecrolimus and tacrolimus). Reproducible results were obtained which showed enhanced depigmented surface areas and higher amounts of T lymphocytes in placebo-treated test zones compared to active anti-inflammatory treated areas.

Our "in vivo vitiligo induction and therapy model" was used in a different set up in 5 additional vitiligo patients and 1 control patient. The aim of this new set up was to evaluate:

1. The effect of cryotherapy on the skin at the histological level (vitiligo versus control skin).
2. Comparison of different variables (e.g. treatment intervals, frequency of application) of 2 different Koebner induction methods (cryotherapy versus lasertherapy) in a double blind randomized controlled setting. Information obtained from this study will also have clinical/therapeutic implications: best application scheme and choice of depigmenting treatment (bleaching treatment= Koebner induction) in case in patients with treatment resistant and very extensive vitiligo.
3. The effect of moisturizing cream on the Koebnerisation process in a double blind randomized setting.

ANALYSES:

We could demonstrate again that our *in vivo* vitiligo induction model is very informative to investigate vitiligo induction and to determine the efficacy of topical treatments in vitiligo. Information was collected related to the effect of moisturizing cream and details the depigmentation process.

8. Safety

No serious adverse events have occurred during the study.

9. At 2 biopsy sides a small hypertrofic scar was visible during follow up. These scars were treated with intralesional steroid with successful result. Some hyperpigmentation was noticed that in general improved during follow up

10. Protocol deviations

No protocol deviations have occurred during the study.

11. Discussion and overall conclusions

We could demonstrate that our *in vivo* vitiligo induction model is very informative to investigate vitiligo induction and to determine the efficacy of topical treatments in vitiligo.

12. References

van Geel N, et al. In vivo vitiligo induction and therapy model: double-blind, randomized clinical trial. Pigment Cell Melanoma Res. 2012 Jan;25(1):57-65.

van Geel N, Depaepe L, Speeckaert R. Laser (755 nm) and cryotherapy as depigmentation treatments for vitiligo: a comparative study. J Eur Acad Dermatol Venereol. 2015 Jun;29(6):1121-7.