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(54) Title: NON-INVASIVE METHOD TO PERFORM SKIN INFLAMMATORY DISEASE PHARMACO-GENOMIC STUDIES AND DIAGNOSIS METHOD THEREOF

(57) Abstract: The present invention relates to non-invasive method to perform skin inflammatory disease pharmaco-genomic studies and diagnosis methods thereof. Invention also concerns discriminating biomarkers and genes and in vitro diagnostic methods using said biomarkers.

Non-invasive method to perform skin inflammatory disease pharmaco-genomic studies and diagnosis method thereof

The present invention relates to non-invasive method to perform skin inflammatory disease pharmaco-genomic studies and diagnosis methods thereof and particularly scalp psoriasis. Invention also concerns discriminating biomarkers and genes and in vitro diagnostic methods using said biomarkers.

The diagnosis and management of patients with inflammatory skin disease/disorders remains a very challenging and rewarding aspect of 'core' dermatology practice. Inflammatory skin diseases/ disorders affect men, women and children of all races. They consist in a broad category ranging in severity and etiopathogeny. They include very common dermatoses such as Psoriasis, Eczema, Atopic dermatitis, Acne, Rosacea, but also less frequent or rare diseases such as lichenoïd eruptions or erythrodermia. While these diseases/ disorders are usually not generally perceived to be serious or life threatening, they can significantly impact on the quality of life for sufferers. Appropriate treatment is based on correct diagnosis.

As described in the following, one specific embodiment of the present invention is diagnosis methods of psoriasis and particularly scalp psoriasis.

Psoriasis is a common chronic skin disorder estimated to affect about 2% of the Western population, with the scalp being the most common site of involvement at the onset and throughout the course of the disease (Van de Kerkhof PC, Franssen ME. Psoriasis of the scalp. Diagnosis and management. *Am J Clin Dermatol* 2001; **2**: 159–165; Farber EM, Nall L. Natural history and treatment of scalp psoriasis. *Cutis* 1992; **49**: 396–400.). Indeed, 50% to 80% of patients with psoriasis report scalp psoriasis or concomitant psoriasis of the scalp and the body, leading to a prevalence of scalp psoriasis of 1.5% to 2% in northwestern Europe. For many patients, psoriasis of the scalp is the most difficult aspect of their disease owing to the visibility of lesions.

Psoriasis is a chronic, inflammatory skin disorder, which is thought to have an immune-mediated pathogenesis whereby activated T cells infiltrate the dermis and stimulate cytokines, thus promoting keratinocyte proliferation (Krueger G. The immunologic basis for the treatment of psoriasis with new biologic agents. *J Am Acad Dermatol* 2002; **46**: 1–23.). Scalp psoriasis does not generally result in hair loss, although some increased shedding of telogen hairs and reduction in hair density is common in psoriasis plaques.

In addition, extensive hair loss can occur in the erythrodermic forms of psoriasis, and chronic severe hyperkeratotic scalp psoriasis may induce scarring alopecia (Bardazzi F, Fanti PA, Orlandi C, Chieregato C, Misciali C.Psoriatic scarring alopecia: observations in four patients.Int J Dermatol. 1999 Oct;38(10):765-8.). In addition, the morphology of hair follicles was examined in psoriatic scalp biopsies and compared with normal scalp. In scalp psoriasis the lower outer root sheath and hair matrix were not affected by the psoriatic changes, although there was an irregular expansion in the proximal lower outer root sheath. This area has been characterized, by the presence of keratin K19-containing cells, as the putative stem cell region. (Wilson CL et al.; 4: Br J Dermatol. 1994 Aug;131(2):191-200 "Keratinocyte differentiation in psoriatic scalp: morphology and expression of epithelial keratins").

Another study analysed anagen hair follicles obtained from both healthy (n = 7) and uninvolved psoriatic (n = 4) scalps were segmentally analyzed for proliferative activity using DNA flow cytometry. Hair follicle kinetics were almost equal in either group except for the infundibular portion which exhibited significant increase of S-phase values in psoriatic patients. Maximum proliferation was disclosed within the bulbar segment. This study confirms that cell kinetics behavior of hair follicles from uninvolved scalp of psoriatics compared with those from healthy scalps is altered in the infundibular portion only. (Katsuoka K,et al.6: Dermatologica. 1987;174(3):105-9. "Cell kinetics of the human anagen hair follicle. Flow cytometric studies in healthy and psoriatic subjects").

A definitive diagnosis of scalp psoriasis may be difficult in cases where there is a clinical overlap with seborrhoeic dermatitis. Nevertheless, microscopic examination of the scalp can be used to confirm a diagnosis of scalp psoriasis, because a characteristic histological appearance associated with the condition has been observed, represented by proliferation of parakeratotic cells, sometimes accompanied by leucocyte infiltration (Conti Diaz IA, Civila E, Veiga R. The importance of microscopic examination in the management of esquamative diseases of the scalp. *Mycopathologia* 2002; **153**: 71–75.).Persistent scaly plaques on a bald scalp occasionally require histological examination to exclude Bowen's disease. An underlying mycotic infection or allergic contact dermatitis (although the latter is less frequent) should be ruled out prior to a diagnosis of scalp psoriasis being made (Elewski BE. Clinical diagnosis of common scalp disorders. *J Invest Dermatol Symp Proc* 2005; **10**: 190–193.; Larko O. Problem sites: scalp, palm and sole, and nail. *Dermatol Clin* 1995; **13**: 771–777.).

According to this prior art none pharmaco-genomics investigations have studied skin inflammatory lesions from hair samples, collecting by non-invasive method, including the scalp psoriatic lesions. The present invention provides a non-invasive method to perform skin

inflammatory disease pharmaco-genomic studies and particularly scalp psoriasis and a diagnose method thereof. Invention also concerns discriminating biomarkers and gene and in vitro diagnostic methods using said biomarkers.

Inventors have found a method to perform non invasive pharmaco-genomic studies which is applicable to affected skin including the scalp for any type of disease, and for any type of pharmacological agent (small molecule drugs; biologics) and any type of application (topical and systemic) as well.

Therefore, in one embodiment, the present invention regards a method to perform non invasive pharmaco-genomic studies of affected skin and/or scalp comprising collecting hair follicles non invasively and analysing gene expression profiling.

Within this study, changes in gene transcription in hair follicles were investigated (collected by a non-invasive method) .

By hair it is meant all kind of hair present on the body skin including scalp hair.

It is meant by non invasive method any method which does not require surgical procedures.

In a specific embodiment, the present invention regards a method to perform non invasive pharmaco-genomic studies of affected scalp comprising collecting hair follicles non invasively and analysing gene expression profiling. Particularly, the method can be used to assess at least one these topics:

i) identify genes allowing to discriminate affected samples to healthy volunteers samples,

ii) identify early markers monitoring a compound or drug efficacy,

iii) characterize compound or drug anti-inflammatory mechanism,

iv) clusterize responders versus non-responders based on large scale gene expression profiling.

In a particular embodiment of the invention, a study was conducted to investigate changes in gene transcription in hair follicles (collected by a non-invasive method) of the psoriatic scalps being treated with clobex 0.05%, versus healthy volunteers.

For instance, the hair follicles are plucked with tweezers from the scalp. The gene expression profiling (real time PCR and large scale gene expression array) is used in an effort to i) identify genes allowing to discriminate affected samples to healthy volunteers samples, ii) identify early markers monitoring Clobex 0.05% efficacy, iii) characterize its anti-inflammatory mechanism, iv) clusterize responders versus non-responders based on large scale gene expression profiling.

In another embodiment, the present invention concerns a non–invasive diagnosis method of inflammatory skin disease or disorders and in a specific embodiment diagnoses psoriasis on scalp comprising the steps of:

- collect non-invasively hair follicles
- study/determine the gene expression by analysing method
- clusterize control samples to skin affected patient(s') samples (specific embodiement psoriatic samples) based discriminating genes.

It is understood by "control" samples, samples (hairs samples) collected from subject(s) in healthy conditions or in non involved inflammatory skin conditions.

In a preferred embodiment, the said non–invasive diagnose method of inflammatory skin disease or disorders comprises discriminating genes/ markers (including proteins) which are selected from the well known inflammatory specific genes and/or markers. Particularly, the discriminating genes/markers are selected from the following:
Keratin 16 (KRT16); gap junction protein, beta 2, (connexin 26) (GJB2); chitinase 3-like 2 (CHI3L2); interleukin 8 (IL8); fatty acid binding protein 5 (FABP5); interleukin 1, beta (IL1B); signal transducer and activator of transcription (STAT1); heparanase (HPSE); solute carrier family 6 (amino acid transporter), member 14 (SLC6A14); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); tumor necrosis factor (TNF); interleukin 1 family, member 5 (delta) (IL1F5); small proline-rich protein 2D (SPRR2D); kallikrein 13 (KLK13); chemokine (C-X-C motif) ligand 10 (CXCL10); desmoglein 3 (pemphigus vulgaris antigen) (DSG3); S100 calcium binding protein A12 (S100A12); interleukin 1 receptor antagonist (IL1RN); superoxide dismutase 2, mitochondrial (SOD2); keratin 6C; (KRT6E); interferon-induced protein with tetratricopeptide repeats 3 (IFIT3); desmocollin 2 (DSC2); endothelial cell growth factor 1 (platelet-derived) (ECGF1); RAS guanyl releasing protein 2 (calcium and DAG-regulated) (RASGRP2); wingless-type MMTV integration site family, member 5A (WNT5A); myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) (MX1); small proline-rich protein 1A (SPRR1A); defensin, beta 4 (DEFB4); S100 calcium binding protein A9 (S100A9); interleukin 1 family, member 9 (IL1F9); kallikrein 6 (neurosin, zyme) (KLK6); matrix metallopeptidase 9 (MMP9); serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPINB3); interferon, gamma (IFNG); lipocalin 2 (oncogene 24p3) (LCN2); interferon, alpha-inducible protein 27 (IFI27); peroxisome proliferator-activated receptor delta (PPARD); serpin peptidase inhibitor, clade B (ovalbumin), member 1 (SERPINB1); latent transforming growth factor beta binding protein 1 (LTBP1); pre-B-cell colony enhancing factor 1 (PBEF1); transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase) (TGM1); chemokine (C-C motif) ligand 20

(CCL20); aldo-keto reductase family 1, member B10 (aldose reductase) (AKR1B10); S100 calcium binding protein A7 (S100A7).

In a specific embodiment , the said non–invasive diagnose psoriasis method comprises discriminating genes which are selected from the following: interleukin 8 (IL8); beta 4defensin (DEFB4); S100 calcium binding protein A7 (S100A7); S100 calcium binding protein A9 (calgranulin B) (S100A9); S100 calcium binding protein A12 (S100A12); interleukin 1b (IL-1b); lipocalin 2 (oncogene 24p3) (LCN2); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); Interferon alpha-inducible protein 27 (IFI27); Peroxisome proliferator-activated receptor- δ (PPAR-δ); serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPIN B3).

In the context of the present invention « gene » refers to nucleic acid or nucleotide sequence encoding for a protein/biomarker expression, and the proteins related to the said gene.

In addition, the present invention relates to the said gene expression product as “biological targets”. By target it is understood an enzyme, a receptor, other protein or mRNA that can be modified by an external stimulus. The definition is context-dependent and can refer to the biological target of a pharmacologically active drug compound, or the receptor target of a hormone. The implication is that a molecule is "hit" by a signal/stimulus and its behavior is thereby changed.

In the context of invention, target of interest are those above mentioned expression products.

By analysing method it is meant any method carried out to determine gene expression levels. Those are generally well known by one skilled in the art and are determined according to transcription or translation rates. By transcription rate it is understood, mRNA levels. By translation it is meant, protein production rate.

Gene expression products/ Biomarkers (e.g. Proteins) might be determined by any appropriate methods such as western-blot, IHC, MAS spectrometry analysis (MAldi-TOF and LC/MS analysis), Radioimmunoassay (RIA), Elisa or by any other methods well known by skilled in the art or by mRNA dosage by any appropriate methods well known by skilled in the art.

For instance, it can be mentioned quantitative or semi-quantitative methods for mRNA of gene of interest detection are well known by one skilled in the art.

Methods based on mRNA hybridation with nucleic probes are typically known (Northern Blot, RT-PCR, RNase protection). It might be advantageous to use detection markers such as fluorescent, radio-labelled, enzymatic agents or other ligands (for example avidine/biotine).

Gene translation rate may also be assessed by immunological assays of gene expression product. To this aim, polyclonal or monoclonal antibodies may be used. Antibodies manufacturing methods are well known by one skilled in the art. For instance, monoclonal antibody might be produced according to Kôhler and Milstein method (Nature (London), 256: 495- 497 (1975) or by cloning a nucleic acid expression clone in hybridoma.
Immunological dosages are assessed by solid or homogeny phase, in one or two time frames; with the so-called sandwich method or with competition method.
In a preferred embodiment, the determination technique is Real time PCR.

Another embodiment of the invention relates to the monitoring of efficacy of a pharmacological agent in preventing or treating inflammatory skin disease/disorders (in a specific embodiment scalp psoriasis) comprising the steps of:

- administrate to a patient in need of treatment a therapeutically effective amount of a pharmacological agent
- collect non-invasively hair follicles
- study/determine the gene expression by analysing method
- Analyse skin affected patient(s') samples to controls' samples or to previous skin affected patient(s') samples without pharmacological agent

The pharmacological agent is selected from a small molecule drug or a biological agent.

The present invention also embodies a method to monitor skin (or scalp in a specific embodiment) inflammation in a skin affected patient(s) (in a specific embodiment psoriatic patient) comprising the steps of:

- collect non-invasively patient's hair follicles
- study/determine the gene expression by analysing method
- Analyse inflammation gene (s) from said inflammatory skin affected patient(s') samples (specific embodiment patient's psoriatic samples) to controls' samples or to previous patient psoriatic samples.

Another embodiment of the invention relates to a predictive model of inflammatory skin affected patient(s') (in a specific embodiment psoriatic scalp) determination comprising monitoring the modulation in expression of selected discriminating biomarkers/genes.
By "modulation in expression" it is meant a change in the expression of selected genes and/or said biomarkers/gene expression products levels and/or their activities in comparison with healthy volunteers and encompasses either a down regulation/under expression or up regulation/over expression.

By analysing method it is meant any method carried out to determine gene expression levels. Those are generally well known by one skilled in the art and are determined according to transcription or translation rates. By transcription rate it is understood, mRNA levels. By translation it is meant, protein production rate.

Gene expression products/ Biomarkers (e.g. Proteins) might be determined by any appropriate methods such as western-blot, IHC, MAS spectrometry analysis (MAldi-TOF and LC/MS analysis), Radioimmunoassay (RIA), Elisa or by any other methods well known by skilled in the art or by mRNA dosage by any appropriate methods well known by skilled in the art.

In a preferred embodiment modulation of at least 1 discriminating genes or markers selected from the inflammatory markers are monitored. The discriminating genes or markers are preferentially selected from the following: interleukin 8 (IL8); beta 4defensin (DEFB4); S100 calcium binding protein A7 (S100A7); S100 calcium binding protein A9 (calgranulin B) (S100A9); S100 calcium binding protein A12 (S100A12); interleukin 1b (IL-1b); lipocalin 2 (oncogene 24p3) (LCN2); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); Interferon alpha-inducible protein 27 (IFI27); Peroxisome proliferator-activated receptor-δ(PPAR-δ); serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPIN B3).

In the context of the invention, it is understood as biomarker a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (NIH definition).
Therefore, biomarkers are used to indicate or measure a biological process (for instance, levels of a specific protein in blood or fluids, genetic mutations, or abnormalities observed in tests). Detecting biomarkers specific to a disease can aid in the identification, diagnosis, and treatment of affected individuals and people who may be at risk but do not yet exhibit symptoms.

Hence, another embodiment of the invention is the inflammatory skin diseases/disorders lesions biomarkers and/or gene expression products (including proteins) as biomarkers selected from the following:
Keratin 16 (KRT16); gap junction protein, beta 2, (connexin 26) (GJB2); chitinase 3-like 2 (CHI3L2); interleukin 8 (IL8); fatty acid binding protein 5 (FABP5); interleukin 1, beta (IL1B); signal transducer and activator of transcription (STAT1); heparanase (HPSE); solute carrier family 6 (amino acid transporter), member 14 (SLC6A14); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); tumor necrosis factor (TNF); interleukin 1 family, member 5 (delta) (IL1F5); small proline-rich protein 2D (SPRR2D); kallikrein 13 (KLK13);

chemokine (C-X-C motif) ligand 10 (CXCL10); desmoglein 3 (pemphigus vulgaris antigen) (DSG3); S100 calcium binding protein A12 (S100A12); interleukin 1 receptor antagonist (IL1RN); superoxide dismutase 2, mitochondrial (SOD2); keratin 6C; (KRT6E); interferon-induced protein with tetratricopeptide repeats 3 (IFIT3); desmocollin 2 (DSC2); endothelial cell growth factor 1 (platelet-derived) (ECGF1); RAS guanyl releasing protein 2 (calcium and DAG-regulated) (RASGRP2); wingless-type MMTV integration site family, member 5A (WNT5A); myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) (MX1); small proline-rich protein 1A (SPRR1A); defensin, beta 4 (DEFB4); S100 calcium binding protein A9 (S100A9); interleukin 1 family, member 9 (IL1F9); kallikrein 6 (neurosin, zyme) (KLK6); matrix metallopeptidase 9 (MMP9); serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPINB3); interferon, gamma (IFNG); lipocalin 2 (oncogene 24p3) (LCN2); interferon, alpha-inducible protein 27 (IFI27); peroxisome proliferator-activated receptor delta (PPARD); serpin peptidase inhibitor, clade B (ovalbumin), member 1 (SERPINB1); latent transforming growth factor beta binding protein 1 (LTBP1); pre-B-cell colony enhancing factor 1 (PBEF1); transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase) (TGM1); chemokine (C-C motif) ligand 20 (CCL20); aldo-keto reductase family 1, member B10 (aldose reductase) (AKR1B10); S100 calcium binding protein A7 (S100A7).

In a specific embodiment, the invention relates to psoriatic scalp lesions biomarkers and/or gene expression products (including proteins) as biomarkers selected from the following: interleukin 8 (IL8); beta 4defensin (DEFB4); S100 calcium binding protein A7 (S100A7); S100 calcium binding protein A9 (calgranulin B) (S100A9); S100 calcium binding protein A12 (S100A12); interleukin 1b (IL-1b); lipocalin 2 (oncogene 24p3) (LCN2); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); Interferon alpha-inducible protein 27 (IFI27); Peroxisome proliferator-activated receptor-δ(PPAR-δ); serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPIN B3).

In another embodiment, the invention concerns an In vitro screening method of pharmacological agent/ drug candidates (or family lead compound) susceptible of preventing and/or treating inflammatory skin diseases/disorders as well as scalp psoriasis associated comprising determine the capacity of said pharmacological agent to modulate e. g. down regulated or up regulate) expression of said selected gene(s) expression and/or said biomarker (s)/gene expression product(s) levels or activity.

In a specific embodiment, the invention is an in vitro screening method of drug candidates susceptible of preventing and/or treating inflammatory skin diseases/disorders; said method comprising the following steps:

a. Collecting at least two biological samples : one mimics pathological skin inflammatory lesion condition and the other mimics healthy condition;

b. Contacting at least one sample or a mixture of samples with one or more drug candidates to be tested;

c. Measuring gene expression or gene expression product level or activity in the biological samples or mixture obtained in b);

d. Selecting drug candidates which are capable of modulating gene expression or gene expression product level or activity measured in said samples or mixture obtained in b) and comparing the levels with a control sample, ie not mixed with drug candidate.

By “modulate” it is understood any effect on expression or activity of biomarkers/gene expression products, any effect on genes or on activity of at least one of their expression promoter(s) and preferentially any effect inducing e. g. a down regulation or an up regulation, a stimulation, an inhibition, totally or partially.

In the context of the present invention and without particular notice, it is understood that «expression of biomarkers/gene expression product » refers to a quantity of a protein or any else product resulting from the transcription and/or translation of a gene. By activity it is meant biological activity.

Description of figures and tables:

Figure 1: RNA extraction from healthy volunteers and scalp psoriasis patients. **a** an active edge of psoriasis lesion on scalp; **b** hair follicles of telogen and anagen phases, with epithelial sheath intact (I), absent (II), or close to intact (III) **c** Representative chromatograms of micro-capillary electrophoresis of RNA collected from hair follicles of a healthy volunteer and a scalp psoriasis patient at three study visits (Baseline, Week 2 and 4).

Figure 2: Total severity score (**a**) and Transcriptomic score (**b**) of patients at Baseline, Week 2 and Week 4 of clobetasol propionate shampoo treatment. (**c**) Average of transcriptomic score and TSS at Baseline, Week 2 and Week 4.

Table 1: List of 10 psoriasis disease-related genes that were significantly up-regulated in hair follicles of scalp psoriasis patients compared to healthy volunteers. Numbers in the column of “Fold increase in psoriasis skin” were extracted from Zhou X et al.[18] except those of S100A7 and PPARδ, which were from Quekenborn-Trinquet V et al.[19] Numbers in the column of “Fold increase in psoriasis hair follicles” and “p value” were results of this study. The p value of the significance of fold induction was calculated by t-test.

Table 2:Correlation between patients' clinical severity scores and transcriptomic score during clobetasol propionate shampoo treatment. For each clinical parameter, an "ok" variable was set to 1 if the clinical score and the transcriptomic score moved in the same direction, and set to 0 if the clinical score and the transcriptomic score moved in the opposite direction. The P value corresponded to the null hypothesis that the agreement probability P(ok=1) is 1/2.

Table 3: Fold modulation of 10 selected psoriasis disease-related genes in healthy volunteers and scalp psoriasis patients. For each gene, the average fold induction of gene expression in hair follicles of scalp psoriasis patients compared to healthy volunteers (psoriatic/healthy) was calculated. The transcriptomic score is the average fold induction of the 10 selected genes in each subject compared to the mean expression level in all healthy volunteers.

Table Supplementary 1 Quality and quantity of RNA collected from hair follicles of scalp psoriasis patients and healthy volunteers.

Table Supplementary S2: List of 44 genes reported to be associated to skin psoriasis and tested in TLDA in this study. Numbers in the column of "Fold increase in psoriasis skin" were extracted from Zhou X et al.,[18] except those marked with *, which were from Quekenborn-Trinquet V et al.[19] Numbers in the column of "Fold increase in psoriasis hair follicles" and "p value" were results of this study. p value was calculated by t-test.

The present invention will be more precisely describes through the following example.

Example :

Non-invasive Gene expression profiling in psoriatic scalp hair follicles: Clobetasol propionate shampoo 0.05% normalizes psoriasis disease markers

The objective of this example is to determine whether psoriasis-related genes are differentially regulated in the hair follicles of scalp psoriasis patients and whether the modulation of these genes can be correlated with clinical severity scores.

Psoriasis is a common and chronic inflammatory disease estimated to affect about 2% of the Western population, with scalp being the most common site of involvement at the onset and throughout the course of the disease.[1] It is an immune-related disorder, triggered

by activated T cells which infiltrate the dermis and stimulate hyperproliferation of keratinocytes.[2] Topical medication remains the most frequent treatment for scalp psoriasis in patients of all severity groups. Among the available treatments, clobetasol propionate shampoo was demonstrated to be effective and safe for patients with moderate or severe scalp psoriasis.[3-6] It was designed to integrate a super potent corticosteroid (clobetasol propionate 0.05%) into a once-daily, short-contact shampoo formulation, in order to minimize the risk of adverse events associated with steroids usage, without compromising efficacy. It was also demonstrated that treatment with clobetasol propionate shampoo improved the patients' quality of life and resulted in high satisfaction[7].

Large scale gene expression profiling has been widely used in the field of dermatology to elucidate the mechanisms of various diseases including psoriasis.[8-9] Gene expression profiling using DNA microarray technology allows simultaneous examination of the expression levels of all human genes. Studies using peripheral blood mononuclear cells (PBMC) and skin samples harvested by biopsy or non-invasive tape striping technology identified genes which were differentially expressed in psoriatic and normal samples.[10-13] Several of these genes, such as the S100 calcium-binding proteins and Defensins, mapped to known disease-associated loci and were previously shown to be up-regulated in psoriatic lesions.[14-15] Recently identified new markers include genes involved in the Wnt pathway, disease-related cytokines and chemokines.[16-18] Changes in disease-related gene expression levels were demonstrated to be correlated with stages of disease progression and clinical severity scores,[19-24] allowing it to be used for examining the effects of treatment. As examples, therapeutic antibodies against tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ), as well as the immune-modulatory drug pimecrolimus were shown to be effective in treating psoriasis.[25-26]

Although direct evidence is not available, several observations suggest that in scalp psoriasis patients, the hair follicles could be affected by the disease. First, although scalp psoriasis does not generally result in hair loss, extensive scarring alopecia can be induced in severely affected scalp areas.[27] Second, an irregular expansion in the proximal lower outer root sheath of hair follicles was observed in psoriatic biopsy samples, when compared to those of normal skin. This area, from which follicle regeneration occurs during the anagen phase of hair growth, was considered as putative stem cell region due to the presence of keratin 19-containing cells.[28] Finally, when the growth kinetics of anagen hair follicles was measured using DNA flow-cytometry, the infundibular portion of the psoriatic hair sample demonstrated a significant increase of proliferation activity in S-phase compared to samples from healthy scalps.[29]

We hypothesized, that scalp psoriasis does not only affect hair follicle growth parameters, but also leads to modulation of psoriasis disease-related genes. To test this hypothesis, we generated a medium scale gene expression profile using RNA extracted from hair follicles of scalp psoriasis patients and healthy volunteers. We also attempted to study the effect of clobetasol propionate shampoo treatment on regulation of these genes and to correlate the disease-related gene expression levels with clinical severity scores of scalp psoriasis patients.

METHODS AND MATERIALS

This study was conducted in accordance with the Declaration of Helsinki, its amendments, Good Clinical Practice and local regulatory requirements including ethics board review. All patients provided written informed consent before entering into the study.

Study design and patient selection

This study was part of a single arm, open study, which comprised the preliminary phase of a double-blind, multi-centre and controlled study on the maintenance effect of clobetasol propionate shampoo. 59 patients were recruited in three centres of Canada for this part of the study. The recruited patients were 18 years or older, with “moderate” or “severe” scalp psoriasis based on their Global Severity Score (GSS) assessment (GSS = 3 or 4 on a scale of 0 to 5, with 0=clear and 5=very severe).

Treatment and clinical assessments

All patients received clobetasol propionate shampoo 0.05% (Clobex® shampoo, Galderma Laboratories, LP, Fort Worth, TX, U.S.A) for up to 4 weeks. The study drug was applied once daily by patients in a thin film onto dry affected scalp areas and left in place for 15 minutes before lathering and rinsing.

The study visits were conducted at Baseline, Week 2 and Week 4. At each visit, the investigators assessed various clinical severity parameters, including erythema (E), scaling (S), plaque thickening (P) (all on a scale of 0 to 4, with 0=none and 4=very severe), extent of disease (Ex) (on a scale of 0 to 5, with 0=none and 5= 80-100%) and GSS. Patients were also asked to indicate their level of pruritus (on a scale of 0 to 3, with 0 = none to 3 = severe) at each visit. At the end of study, Total Severity Score (TSS) was calculated as TSS=E+S+P, and Modified Psoriasis Area and Severity Index (MPASI) was calculated as MPASI= (E+S+P)*Ex.[30]

Sample collection and RNA exaction

Hair samples were collected from the recruited patients at each study visit. A minimum of 15 anagen phase hair follicles were plucked from the active edge of psoriatic lesions. The hair follicles with a bulb and an intact or close-to-intact sheath were subsequently processed. Plucked anagen hair follicles from 8 healthy volunteers were also included for analysis.

Hair shafts were cut 1-2mm above the dermal sheath and dissolved in 500µl RLT buffer (Qiagen Inc.) with 10µl/ml β-mercaptoethanol. Total RNA was extracted using RNeasy extraction kits (Qiagen Inc.) according to manufacturer's protocol. RNA Quantity was measured using Quant-it RNA assay kit (Molecular Probes) and the quality was monitored by following the electrophoresis behaviour of RNA using a 2100 Bioanalyser (Agilent). 50ng of extracted RNA of good quality [RNA indication number (RIN) ≥ 7] and a minimum concentration of 4ng/µl was then used for synthesizing cDNA using high capacity cDNA archive kits (Applied Biosystems).

TaqMan low density array (TLDA) analysis

A single TLDA array contains 8 replicates of the PCR primers for 48 genes (44 selected genes of interest and 4 housekeeping genes). A single determination was performed for samples from scalp psoriasis patients (SP), while samples from healthy volunteers (HV) were analyzed in duplicates.

Synthesized cDNA was added to the PCR master mix, and the mixture was loaded by centrifugation into the wells of the array containing the lyophilized primer sets (Applied Biosystems). The wells were sealed and the reactions were conducted on ABI 7900HT (Applied Biosystems). PCR threshold cycle (Ct) numbers at which the fluorescent signal of the generated nascent DNA exceeds a threshold value was determined. The Ct number was normalized by first subtracting the average Ct of the housekeeping genes in the same sample, and then adding back the average Ct of the housekeeping genes across all samples.

Statistical analysis

The fold modulation of gene expression of scalp psoriasis samples versus samples of healthy volunteers was defined as $2^{(\text{mean CtHV} - \text{mean CtSP})}$, with Ct_{HV} and Ct_{SP} depicting the Ct values of healthy volunteer and scalp psoriasis samples, respectively. One-way ANOVA with Benjamini-Hochberg multiplicity correction was performed using JMP 7.0.1 (SAS Institute) and irMF 3.5 (National Institute of Statistical Sciences, NISS) software, in order to identify genes that were significantly modulated in scalp psoriasis samples.

To assess the correlation between the transcriptomic score and clinical severity scores, an "ok" variable was created and defined as follows: the variable was set to 1 if transcriptomic score and clinical score change toward the same direction; otherwise the variable was set to 0. The p value of the analysis corresponded to the null hypothesis that the agreement probability $P_{ok=1}$ is 0.5.

RESULTS

Inflammation-related genes are up-regulated in scalp hair follicles of psoriasis patients

To determine whether hair follicles are affected by scalp psoriasis, we generated gene expression profiles using RNA extracted from hair follicles of both scalp psoriasis patients and healthy volunteers. Hair samples were collected at Baseline, Week 2 and 4 of treatment with clobetasol propionate shampoo. A minimum of 15 anagen phase hair follicles were plucked from the active edge of psoriatic lesions (Fig 1a). Only hair follicles with a bulb and an intact or close-to-intact sheath were processed (Fig 1b, I and III). Hair plucking caused only mild discomfort to patients and volunteers, and did not induce Koebner phenomenon when performed at sites of remission. Quality of RNA extracted from hair follicles was evaluated by micro-capillary electrophoresis and representative chromatograms are shown in Fig 1c. For all RNA samples, the 18S, 28S and 5S ribosomal RNA peaks were clearly visible, with no degradation detected. RIN, an indicator of RNA quality, was calculated for each sample. Extracted RNA from all 8 healthy volunteers and from 31 of 59 patients had a RIN of 7 or higher, adequate for RT-PCR analysis.[31] The concentration of extracted RNA was variable among samples, but nevertheless all fulfilled the minimum requirement for the downstream procedure (Table S1). Taken together, we obtained RNA of good quality and sufficient quantity for gene expression analysis from both healthy volunteers and scalp psoriasis patients.

The RNA extracted from volunteers and patients was subsequently used for TLDA analysis, a high through-put functional genomics screening technology.[32-34] In scalp hair follicles, we chose to determine the expression levels of 44 genes that were previously reported to be up-regulated in psoriasis skin lesions (Table S2). Four housekeeping genes (18S rRNA, β-actin, GAPDH and HPRT1) were also included in the analysis for normalization purposes. Among the 31 samples whose RNA quality and quantity were adequate for TLDA analysis, 28 samples generated data of good quality based on the expression levels of housekeeping genes and were proceeded to statistical analysis. A total of 10 genes were determined to be significantly up-regulated in hair follicles of scalp psoriasis patients compared to healthy volunteers (≥1.8 fold induction on average with $p \leq 0.01$) (Table 1). These 10 genes were reported to be modulators of the inflammatory response, or to be up-

regulated under inflammatory conditions, indicating that the hair follicles of scalp psoriasis patients were affected by inflammation.

The heat map showing the modulation of the 10 genes is depicted in Fig 2. The genes were arranged from left to right according to the average fold induction of expression level in hair follicles of scalp psoriasis patients versus healthy volunteers. To arrange subjects, a transcriptomic score was defined as the average fold induction of the gene expression level in each subject compared to the mean level in all healthy volunteers. When the subjects were arranged based on their transcriptomic scores, all scalp psoriasis patients had a score equal to or higher than 2 and clustered in a distinct group, except one patient which was inserted among the healthy volunteers, indicating that the transcriptomic score can be considered as a molecular indicator of disease severity.

Clobetasol propionate shampoo is effective in decreasing both transcriptomic score and severity of scalp psoriasis

Recruited scalp psoriasis patients received daily treatment with clobetasol propionate shampoo, and the effect of treatment was evaluated by transcriptomic score and various clinical assessments. After 4 weeks of daily treatment, the mean GSS decreased from 3.5±0.5 to 1.8±0.8, the mean MPASI decreased from 21.1±12.3 to 5.3±6.0 and the mean TSS decreased from 7.8±1.4 to 3.2±1.8 (Fig 3a). Correspondingly, pruritus, extent of the disease and individual sign scores including erythema, scaling and plaque thickening improved after treatment (data not shown). The transcriptomic score decreased after 2 or 4 weeks of treatment as well (Fig 3b). Therefore, the treatment of clobetasol propionate shampoo induced a strong and progressive decrease in both transcriptomic score and clinical severity score such as TSS (Fig 3c), suggesting that the treatment was effective not only in improving the scalp psoriasis lesion conditions, but also in relieving the inflammatory response.

To determine whether the transcriptomic score and clinical severity scores are correlated, we examined whether these scores change towards the same direction upon treatment with clobetasol propionate shampoo. As shown in Table 2, skin phototype as an intrinsic parameter of each patient, remained unchanged upon treatment and therefore was not correlated with the transcriptomic score. Neither was extent of the disease correlated with transcriptomic score during the treatment. However, the other clinical severity scores examined, including MPASI, GSS, TSS, pruritus, erythema, scaling and plaque thickening, all demonstrated a significant correlation after 4-weeks of treatment. The correlation was strong but less significant when assessed after two weeks of treatment. Taken together,

these results suggested that the transcriptomic score is a suitable molecular and local indicator for the clinical severity of scalp psoriasis.

For the first time and as shown in figure 2, it is demonstrated that scalp psoriasis is associated to significant effects on gene expression in plucked hair follicles. So, hair follicles collected by a non invasive method can be used to monitor skin or scalp inflammation.

Using the predictive model, we monitored the effects of Clobex shampoo 0.05% on a molecular level, by a non-invasive method in hair follicles of the psoriatic scalp.

As figured in Figure 3, it is shown the clobex shampoo efficiency following expression of 12 discriminating genes in hair follicles of the psoriatic scalp collected by a non-invasive method.

DISCUSSION

In the present study, we demonstrated that 10 inflammation-related genes were significantly up-regulated in the hair follicles of scalp psoriasis patients. We defined the transcriptomic score as the mean fold modulation of the expression level of the 10 genes, showed that the score indicated severity of the disease on a molecular level and that it correlated with various clinical assessments, including GSS, TSS and MPASI. Clobetasol propionate shampoo treatment, which was demonstrated to be effective in treatment of the clinical signs of scalp psoriasis, also led to a decrease of the transcriptomic score.

Although studies using skin biopsy have generated valuable information on psoriasis, the invasive nature of the technique render it impractical as a routine method for monitoring the disease progression or pharmacogenomic effects of various treatments. A tape-striping method was developed and utilized to harvest RNA for gene expression profiling; however, it has the drawbacks of requiring large skin surface areas and produces only low RNA yield, particularly in healthy volunteers.[13, 35-36] We validated in this study that hair follicle plucking is suitable for collecting RNA of good quality and sufficient quantity for gene expression profiling. Therefore, this minimally invasive technique can be used to diagnose scalp psoriasis and to study the mechanism of the disease.

We demonstrated for the first time that the scalp hair follicle cells were affected by psoriasis. The 44 genes, whose expression level in hair follicles was examined in this study, were selected based on their reported elevated expression level in skin or blood samples harvested from psoriasis patients.[18-19] These genes were known to be involved in various biological pathways, including inflammation, immune response, proliferation and

differentiation of the epidermis. The 10 genes, which were demonstrated to be significantly over-expressed in hair follicles of scalp psoriasis patients, are all functionally related to inflammation. They either act as regulators or mediators of inflammation (IL-8,[37] LCN2,[38] PBEF1, [39] HPSE,[40] DEFB4[41] and three members of the S100 protein family, S100A7,[42] S100A9[43] and S100A12[44]) or are up-regulated under inflammatory conditions (IF127).[45] PPARδ was demonstrated to be involved in both modulation of inflammation and proliferation of keratinocytes.[46] It was also shown to enhance keratinocyte proliferation in psoriasis.[47] Based on the reported functions and the observed up-regulation of these genes, we conclude that the hair follicles of scalp psoriasis patients are affected by inflammation. Clobetasol propionate shampoo was demonstrated to be effective and safe in treatment of moderate to severe scalp psoriasis, since its usage results in lower scores of individual signs and global assessments.[3-6] In this study, we demonstrated that clobetasol propionate shampoo also led to a decrease of patients' transcriptomic scores, thus the treatment down-regulated the genes which were over-expressed under psoriasis conditions. Since all these genes play a role in inflammation, this result strongly suggests that clobetasol propionate shampoo was effective in alleviating the signs of inflammation in scalp hair follicles, confirming the previously reported anti-inflammatory property of corticosteroids.[48] Furthermore, it implicates that the clinical symptoms of scalp psoriasis are at least in part caused by inflammation.

We observed that the transcriptomic score correlated with clinical severity of scalp psoriasis, based on the difference of transcriptomic score among healthy volunteers and psoriasis patients, and on the improvement of both disease severity and transcriptomic score upon clobetasol propionate treatment. However, it should be noted that the present study is based on the analysis of a restricted set of genes previously identified in skin biopsies. Disease markers of psoriatic skin might not be the best choice for genes to be followed in scalp psoriasis; it is therefore likely that a better correlation can be achieved by generating a large scale gene expression profile to identify robust biomarkers of scalps psoriasis, whose change of expression level is then followed. Furthermore, since hair plucking at study visits was not guided with precise localization technique and was instead conducted always at the active edge of psoriatic lesions, it is possible that a slightly different region was sampled each time due to the decrease of disease extent throughout treatment (data not shown). In future studies, precise localization of the plucked areas, as well as local clinical scoring techniques should further improve the correlation between clinical and molecular severity parameters.

Being a local indication, transcriptomic score has its limitation when compared to clinical parameters, which are global assessments. Consistently, the transcriptomic score correlates significantly with individual sign scores, TSS, GSS and MPASI, but not with extent

of the disease. Image-guided hair sampling in several different scalp locations could constitute a solution to this limitation and lead to a more generalized transcriptomic score, which would reflect not only the local clinical severity, but also the extent of the disease.

As costs for development of new drugs rise constantly, while chances of success stagnate, initiatives were launched in US and Europe calling for the development of new tools including biomarkers, that make the drug development process more efficient and effective (The critical path initiative [http://www.fda.gov/oc/initiatives/criticalpath/]; Innovative Medicines Initiative [http://imi.europa.eu/index_en.html]). Furthermore, rules for exploratory investigational new drugs studies have been issued (http://www.fda.gov/cder/guidance/7086fnl.htm). These studies usually involve very limited human exposure and have no therapeutic intent; however, they can determine whether a mechanism of action defined in experimental systems can also be observed in humans, thereby allowing early decision-taking in the development process. The results described in this article set the basis for applying genomic biomarker studies on tiny skin surfaces to test the efficacy of drugs for the treatment of scalp psoriasis.

Thus the present example, demonstrate that the RNA of good quality and sufficient quantity was obtained from hair follicles of psoriasis patients and healthy volunteers. The expression level of 10 inflammation-related genes was significantly increased in psoriatic hair follicles. The patient's transcriptomic score, defined as the mean fold modulation of these 10 genes compared to healthy volunteers, correlated with clinical severity scores. Clobetasol propionate shampoo was effective in decreasing both the transcriptomics and the clinical severity scores.

Hence, hair follicles of scalp psoriasis patients are affected by the inflammatory process. The change of the expression level of inflammation-related genes correlates with the severity of the disease.

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CLAIMS

1. A method to perform non invasive skin inflammatory disease/disorders pharmaco-genomic studies comprising the steps of:
   - collecting hair follicles non invasively
   - analysing gene expression profiling
2. The method according to claim 1, wherein the disease is psoriasis.
3. The method according to claim 1, wherein the hair is collected from the scalp.
4. A method to monitor efficacy of a pharmacological agent in preventing or treating inflammatory skin disease/disorders comprising the steps of:
   - administrate to a patient in need of treatment a therapeutically effective amount of a pharmacological agent
   - collect non-invasively hair follicles
   - study/determine the gene expression by analysing method
   - Analyse skin affected patient(s') samples to controls' samples or to previous skin affected patient(s') samples without pharmacological agent
5. Evaluation efficacy method of a pharmacological agent according to claim 4, wherein the pharmacological agent is selected from a small molecule drug or a biological agent.
6. The method according to claim 4, wherein the disease is psoriasis.
7. The method according to claim 4, wherein the hair is collected from the scalp.
8. A non–invasive diagnosis method of inflammatory skin disease/disorders comprising the steps of:
   - collect non-invasively hair follicles
   - study/determine the gene expression by analysing method
   - clusterize control samples to skin affected patient(s') samples (specific embodiment psoriatic samples) based discriminating genes.
9. The method according to claim 7, wherein the disease is psoriasis.
10. The method according to claim 7, wherein the hair is collected from the scalp
11. The method according to claim 7, wherein the analysing method is Real time PCR
12. The method according to claim 7, wherein discriminating genes are selected in the following: Keratin 16 (KRT16); gap junction protein, beta 2, (connexin 26) (GJB2); chitinase 3-like 2 (CHI3L2); interleukin 8 (IL8); fatty acid binding protein 5 (FABP5); interleukin 1, beta (IL1B); signal transducer and activator of transcription (STAT1); heparanase (HPSE); solute carrier family 6 (amino acid transporter), member 14 (SLC6A14); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1);

tumor necrosis factor (TNF); interleukin 1 family, member 5 (delta) (IL1F5); small proline-rich protein 2D (SPRR2D); kallikrein 13 (KLK13); chemokine (C-X-C motif) ligand 10 (CXCL10); desmoglein 3 (pemphigus vulgaris antigen) (DSG3); S100 calcium binding protein A12 (S100A12); interleukin 1 receptor antagonist (IL1RN); superoxide dismutase 2, mitochondrial (SOD2); keratin 6C; (KRT6E); interferon-induced protein with tetratricopeptide repeats 3 (IFIT3); desmocollin 2 (DSC2); endothelial cell growth factor 1 (platelet-derived) (ECGF1); RAS guanyl releasing protein 2 (calcium and DAG-regulated) (RASGRP2); wingless-type MMTV integration site family, member 5A (WNT5A); myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) (MX1); small proline-rich protein 1A (SPRR1A); defensin, beta 4 (DEFB4); S100 calcium binding protein A9 (S100A9); interleukin 1 family, member 9 (IL1F9); kallikrein 6 (neurosin, zyme) (KLK6); matrix metallopeptidase 9 (MMP9); serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPINB3); interferon, gamma (IFNG); lipocalin 2 (oncogene 24p3) (LCN2); interferon, alpha-inducible protein 27 (IFI27); peroxisome proliferator-activated receptor delta (PPARD); serpin peptidase inhibitor, clade B (ovalbumin), member 1 (SERPINB1); latent transforming growth factor beta binding protein 1 (LTBP1); pre-B-cell colony enhancing factor 1 (PBEF1); transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase) (TGM1); chemokine (C-C motif) ligand 20 (CCL20); aldo-keto reductase family 1, member B10 (aldose reductase) (AKR1B10); S100 calcium binding protein A7 (S100A7).

13. The method according to claim 8, wherein discriminating genes are selected in the following: interleukin 8 (IL8); beta 4defensin (DEFB4); S100 calcium binding protein A7 (S100A7); S100 calcium binding protein A9 (calgranulin B) (S100A9); S100 calcium binding protein A12 (S100A12); interleukin 1b (IL-1b); lipocalin 2 (oncogene 24p3) (LCN2); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); Interferon alpha-inducible protein 27 (IFI27); Peroxisome proliferator-activated receptor- δ(PPAR-δ); serpin peptidase inhibitor, clade B (ovalbumin), member 3(SERPIN B3).

14. A method to monitor skin or scalp inflammation in a skin affected patient comprising the steps of:
    - collect non-invasively patient's hair follicles
    - study/determine the gene expression by analysing method
    - Analyse inflammation gene (s) from said inflammatory skin affected patient(s') samples to controls' samples.

15. A method to monitor skin or scalp inflammation in a psoriatic patient comprising the steps of:
    - collect non-invasively patient's hair follicles
    - study/determine the gene expression by analysing method
    - Analyse inflammation gene (s) from said inflammatory skin affected patient(s') samples to controls' samples or to previous patient psoriatic samples.

16. A predictive model of inflammatory skin affected patient(s') determination comprising monitoring the modulation in expression of at least 1 discriminating genes or markers selected from the inflammatory markers.
17. the predictive model according to claim 16 wherein inflammatory skin affected patient(s') is psoriatic patient..
18. Discriminating genes or markers according to claim 17, selected from the following: interleukin 8 (IL8); beta 4defensin (DEFB4); S100 calcium binding protein A7 (S100A7); S100 calcium binding protein A9 (calgranulin B) (S100A9); S100 calcium binding protein A12 (S100A12); interleukin 1b (IL-1b); lipocalin 2 (oncogene 24p3) (LCN2); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); Interferon alpha-inducible protein 27 (IFI27); Peroxisome proliferator-activated receptor-δ (PPAR-δ); serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPIN B3).
19. Psoriatic scalp lesions biomarkers and/or gene expression products as biomarkers selected from the following: interleukin 8 (IL8); beta 4defensin (DEFB4); S100 calcium binding protein A7 (S100A7); S100 calcium binding protein A9 (calgranulin B) (S100A9); S100 calcium binding protein A12 (S100A12); interleukin 1b (IL-1b); lipocalin 2 (oncogene 24p3) (LCN2); transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1); Interferon alpha-inducible protein 27 (IFI27); Peroxisome proliferator-activated receptor-δ (PPAR-δ); serpin peptidase inhibitor, clade B (ovalbumin), member 3 ?(SERPIN B3).

Table 1

Gene	Name	Function	Fold increase in psoriasis skin	Fold increase in psoriasis hair follicles	p value
IL8	interleukin 8	inflammatory response/ chemotaxis	13.9	29.5	<0.001
DEFB4	defensin, beta 4	immune response / defense response	5.7	20.3	<0.001
S100A7	S100 calcium binding protein A7	epidermis development/ angiogenesis	296	11.1	<0.001
S100A9	S100 calcium binding protein A9 (calgranulin B)	inflammatory response	3.1	6.3	<0.001
S100A12	S100 calcium binding protein A12 (calgranulin C)	inflammatory response	33.6	5.0	0.003
LCN2	lipocalin 2 (oncogene 24p3)	modulator of inflammation	9.9	2.0	0.001
IFI27	interferon, alpha-inducible protein 27	ND	3.5	1.9	0.002
PBEF1	pre-B-cell colony enhancing factor 1	positive regulation of cell proliferation/cell-cell signalling	3.4	1.8	0.004
HPSE	heparanase	proteoglycan metabolic process	4.9	1.8	0.01
PPARδ	peroxisome proliferator-activated receptor delta	fatty acid catabolic and epidermis proliferation	5.5	1.8	0.01

Table 2

Baseline → Week 4			Baseline → Week 2		
Parameter	# ok (total=27)	P value	Parameter	# ok (total=27)	P value
GSS	22	0.0002	GSS	20	0.0030
MPASI	22	0.0002	MPASI	17	0.0610
TSS	22	0.0002	TSS	17	0.0610
Erythema	22	0.0002	Erythema	18	0.0261
Scaling	22	0.0002	Scaling	21	0.0008
Plaque thickening	22	0.0002	Plaque thickening	17	0.0610
Pruritus	19	0.0096	Pruritus	17	0.0610
Extent of disease	16	0.1239	Extent of disease	13	0.5000
Skin phototype	6	0.9970	Skin phototype	9	0.9390

Table 3

Sample	IL8	DEFB4	S100A7	S100A9	S100A12	LCN2	IFI27	PBEF1	HPSE	PPARd	Transcriptomic score
HV1	0,1	0,3	0,7	0,6	0,5	0,7	0,9	0,8	0,9	1,1	0,6
HV2	0,1	0,5	0,9	0,5	0,3	1,3	0,8	1,2	0,9	1,2	0,6
HV3	0,7	0,3	0,8	0,7	0,6	1,0	1,2	1,0	0,7	1,2	0,8
HV4	1,5	0,4	1,0	0,6	0,8	0,8	1,3	1,1	0,5	1,5	0,9
HV5	2,5	2,1	0,8	0,9	0,9	0,6	0,9	1,0	0,9	0,9	1,0
HV6	1,4	2,0	0,9	1,7	1,2	1,0	0,5	1,0	1,1	0,7	1,1
PSO22	0,1	3,8	5,3	1,5	1,5	0,9	1,5	1,3	0,8	1,8	1,3
HV7	1,7	2,8	1,2	3,0	5,7	1,4	1,5	1,1	2,3	0,6	1,8
HV8	8,9	4,9	2,2	1,5	2,7	1,4	1,4	0,9	1,5	1,0	2,0
PSO5	3,4	2,3	3,3	3,1	1,0	2,5	2,0	1,0	1,3	2,3	2,0
PSO20	1,9	3,0	4,0	2,0	2,0	2,0	2,3	1,5	1,5	1,8	2,1
PSO6	14,2	3,2	5,2	1,9	0,8	2,6	2,7	1,1	0,7	1,6	2,3
PSO26	17,6	7,5	5,7	4,3	1,7	1,2	0,9	1,1	1,1	1,0	2,4
PSO2	6,4	1,7	13,7	6,7	2,6	1,9	1,4	1,4	0,9	1,7	2,6
PSO28	22,6	22,2	6,9	3,3	0,7	1,2	1,9	1,2	1,4	0,7	2,7
PSO8	7,6	6,7	7,6	4,0	7,4	0,9	1,2	1,3	1,1	3,0	3,0
PSO13	11,3	13,3	9,1	4,3	0,9	2,1	1,8	1,5	1,4	1,6	3,0
PSO15	3,1	3,3	7,7	2,9	14,3	1,8	1,7	1,2	1,9	3,7	3,1
PSO27	1,7	14,1	10,1	6,2	3,3	1,9	2,3	1,4	3,0	1,5	3,3
PSO3	6,8	5,4	13,8	10,0	3,0	3,5	1,8	1,6	2,0	1,4	3,6
PSO7	71,3	2,9	8,6	7,7	3,6	1,8	1,2	1,4	1,1	4,0	3,8
PSO10	194,4	10,1	7,6	2,7	1,1	1,6	2,8	1,4	1,8	1,6	3,9
PSO17	34,5	32,3	13,0	6,4	4,1	0,9	3,4	1,9	3,4	0,3	4,3
PSO11	25,1	9,3	11,7	7,1	5,8	1,7	2,2	2,4	1,5	3,3	4,7
PSO25	34,6	93,0	7,8	4,8	7,1	1,2	1,8	1,1	2,6	1,8	5,0
PSO24	40,2	11,0	18,4	6,4	3,9	3,3	2,6	1,5	2,5	1,9	5,1
PSO1	16,6	67,3	26,4	9,5	8,1	2,4	1,0	2,1	1,4	1,1	5,3
PSO14	286,0	8,9	15,1	4,1	4,3	2,3	2,1	1,6	3,0	1,5	5,5
PSO16	1,9	12,3	21,6	25,9	10,7	4,8	4,2	4,0	1,0	5,8	6,0
PSO9	31,2	116,9	28,4	10,8	10,2	3,2	1,5	1,9	1,3	1,6	6,8
PSO19	174,9	86,6	15,4	14,1	9,5	5,1	2,0	2,2	2,3	2,1	9,0
PSO12	817,6	375,3	18,3	10,9	13,2	0,6	0,5	5,3	1,7	2,4	9,4
PSO18	612,2	121,5	16,3	14,0	14,7	4,2	1,7	2,2	2,2	0,9	9,8
PSO4	151,4	404,8	16,9	11,1	40,8	2,3	2,0	2,3	4,4	1,9	11,5
PSO21	15856,8	775,8	17,4	16,9	21,8	1,8	1,8	9,6	2,8	2,2	20,7
PSO23	1390,2	729,6	26,9	27,4	324,7	2,8	6,2	4,3	10,3	2,3	29,1
psoriatic /healthy	29,5	20,3	11,1	6,3	5,0	2,0	1,9	1,8	1,8	1,8	

Table Supplementary 1

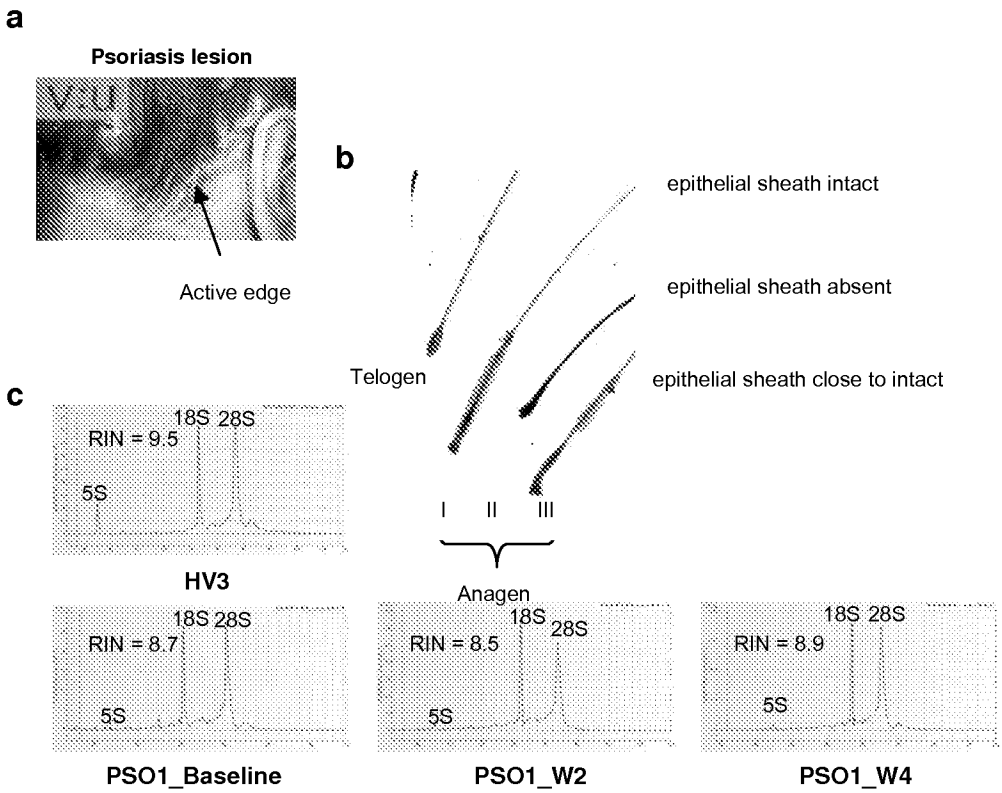
	RIN			Concentration (ng/µl)		
		Clobex daily treatment			Clobex daily treatment	
Sample	Baseline (W0)	Week 2	Week 4	Baseline (W0)	Week 2	Week 4
PSO11	9	9	8	138	45	27
PSO13	8	8	8	186	26	18
PSO23	7	8	9	24	38	6
PSO18	8	9	8	760	1052	1149
PSO4	9	9	9	5	7	6
PSO15	8	7	7	59	37	57
PSO7	9	7	9	261	44	609
PSO19	7	9	9	122	51	15
PSO24	8	7	8	133	82	110
PSO3	8	8	8	111	116	301
PSO14	9	8	7	20	40	41
PSO28	8	8	9	213	137	523
PSO9	9	6	7	486	542	256
PSO22	7	8	7	33	41	164
PSO1	9	9	9	642	907	539
PSO27	7	9	9	55	30	375
PSO20	8	7	9	223	797	149
PSO29	10	9	9	18	210	25
PSO30	9	9	10	30	7	8
PSO16	9	9	9	49	37	26
PSO5	10	8	10	48	97	58
PSO8	9	10	9	75	12	22
PSO2	8	9	7	41	4	32
PSO26	9	9	9	86	100	41
PSO6	9	9	8	195	74	368
PSO10	9	9	9	74	44	35
PSO12	8	9	9	143	171	116
PSO31	9	9	9	46	91	23
PSO21	7	8	9	60	63	12
PSO17	9	9	8	5	357	272
PSO25	9	9	9	366	106	51
HV 1	10			278		
HV 2	10			684		
HV 3	10			369		
HV 4	10			425		
HV 5	10			232		
HV 6	10			348		
HV 7	10			526		
HV 8	10			355		

Table Supplementary S2

Gene	Name	Function	Fold increase in psoriasis skin	Fold increase in psoriasis hair follicles	P value
IL8	interleukin 8	inflammatory response/ chemotaxis	13.9	29.5	<0.001
DEFB4	defensin, beta 4	immune response / defense response	5.7	20.3	<0.001
S100A7	S100 calcium binding protein A7	epidermis development/ angiogenesis	296*	11.1	<0.001
S100A9	S100 calcium binding protein A9 (calgranulin B)	inflammatory response	3.1	6.3	<0.001
S100A12	S100 calcium binding protein A12 (calgranulin C)	inflammatory response	33.6	5.0	0.003
IL1B	interleukin 1, beta	inflammatory response/ apoptosis	9.1*	2.6	0.07
TNFa	tumor necrosis factor alpha	immune response/anti-apoptosis	5.5*	2.1	0.03
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3	Unknown	3.1	2.1	0.03
LCN2	lipocalin 2 (oncogene 24p3)	modulator of inflammation	9.9	2.0	0.001
MMP9	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	extracellular matrix organization and biogenesis	4.2	1.9	0.3
TCN1	transcobalamin I (vitamin B12 binding protein, R binder family)	cobalamin transport	28.1	1.9	0.2
IFI27	interferon, alpha-inducible protein 27	ND	3.5	1.9	0.002
PBEF1	pre-B-cell colony enhancing factor 1	positive regulation of cell proliferation/cell-cell signalling	3.4	1.8	0.004
HPSE	heparanase	proteoglycan metabolic process	4.9	1.8	0.01
PPARD	peroxisome proliferator-activated receptor delta	fatty acid catabolic and epidermis proliferation	5.5*	1.8	0.01
IL1F9	interleukin 1 family, member 9	cell-cell signalling	11.2	1.7	
SERPINB3	serpin peptidase inhibitor, clade B (ovalbumin), member 3	serine-type endopeptidase inhibitor activity	8.9	1.7	
KLK13	kallikrein 13	proteolysis	5.3	1.7	
SOD2	superoxide dismutase 2, mitochondrial	response to oxidative stress	3.2	1.6	
SERPINB1	serpin peptidase inhibitor, clade B (ovalbumin), member 1	serine-type endopeptidase inhibitor activity	5.8	1.4	
ECGF1	endothelial cell growth factor 1 (platelet-derived)	angiogenesis / pyrimidine nucleotide metabolic process	6.5	1.2	

LTBP1	Latent transforming growth factor beta binding protein 1	TGFb signalling	3.4	1.2	
SPRR1A	small proline-rich protein 1A	keratinocyte differentiation	3.1	1.2	
MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	defense response / induction of apoptosis	4.3	1.2	
STAT1	signal transducer and activator of transcription 1, 91kDa	regulation of progression through cell cycle / transcription from RNA polymerase II promoter	3.7	1.1	
KLK6	kallikrein 6 (neurosin,)	collagen catabolic process	4.5	1.1	
TGM1	transglutaminase 1	keratinocyte differentiation	3.4	1.1	
DSG3	desmoglein 3 (pemphigus vulgaris antigen)	cell adhesion	3	1.0	
DSC2	desmocollin 2	cell adhesion	5.1	0.9	
SLC6A14	solute carrier family 6 (amino acid transporter), member 14	amino acid metabolic process	7.4	0.9	
AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)	steroid metabolic process	6.1	0.9	
IL1RN	interleukin 1 receptor antagonist	immune response	3	0.8	
GJB2	gap junction protein, beta 2, 26kDa (connexin 26)	Cell communication	50.5	0.8	
KRT16	keratin 16 (focal non-epidermolytic palmoplantar keratoderma)	epidermis development	3.7	0.8	
FABP5	fatty acid binding protein 5 (psoriasis-associated)	epidermis development	5.3	0.7	
IL1F5	interleukin 1 family, member 5 (delta)	inflammatory response	3.9	0.6	
WNT5A	wingless-type MMTV integration site family, member 5A	Wnt receptor signalling pathway	3.4	0.5	
KRT6E	keratin 6E	cytoskeleton organization and biogenesis	6.8	0.3	
IFNG	interferon, gamma	immune response	5.7*	undetected	
CCL20	chemokine (C-C motif) ligand 20	inflammatory response / chemotaxis	4.5	undetected	
CHI3L2	chitinase 3-like 2	carbohydrate metabolic process	3.6	undetected	
SPRR2D	small proline-rich protein 2D	epidermis development /keratinocyte differentiation	3.3	undetected	
RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	regulation of cell growth / Ras protein signal transduction	16.1	undetected	
CXCL10	chemokine (C-X-C motif) ligand 10	inflammatory response / chemotaxis	22*	undetected	

Figure 1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Psoriasis lesion
Active edge
b
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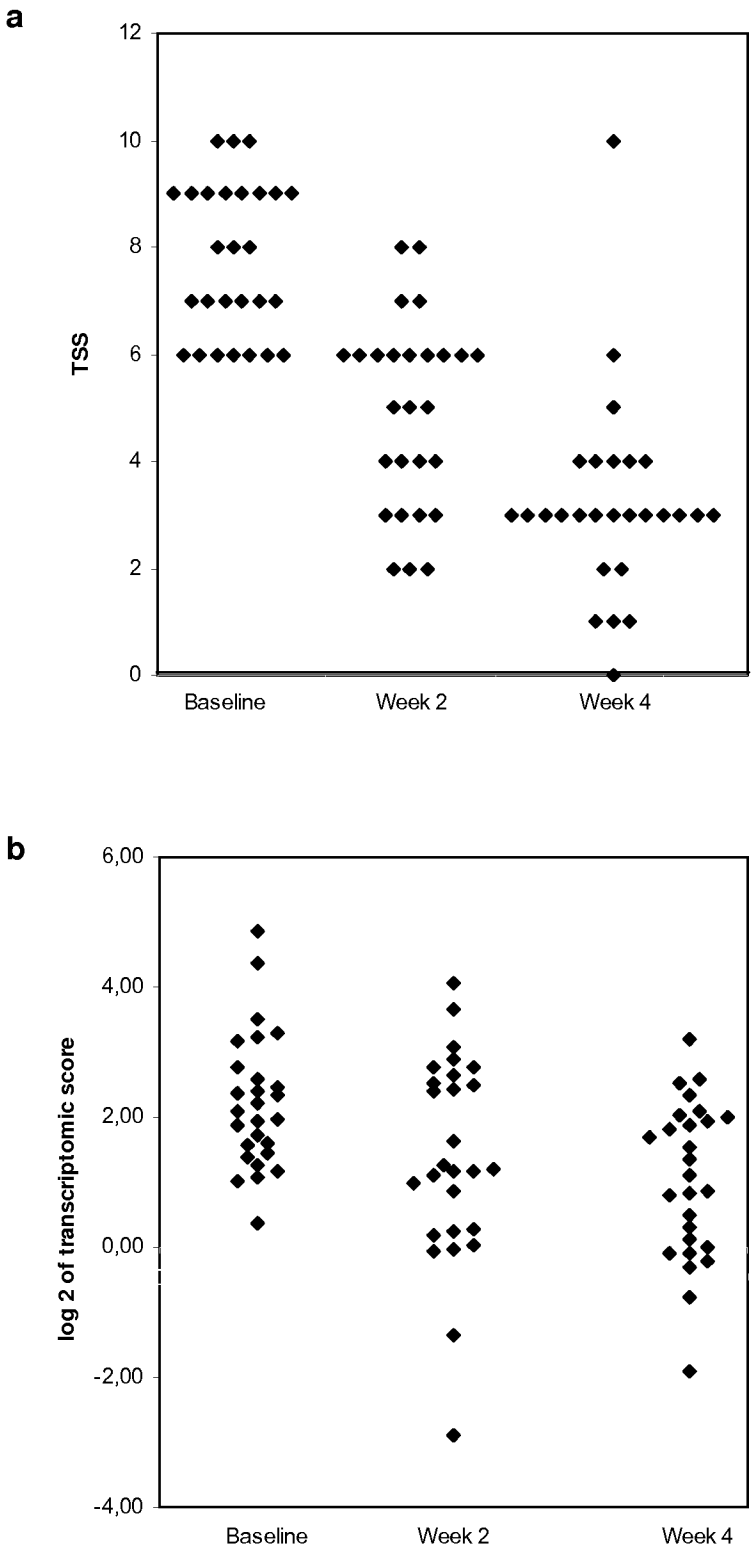
epithelial sheath absent
epithelial sheath close to intact
Telogen
I II III
Anagen
c
18S 28S
RIN = 9.5
5S
HV3
RIN = 8.7
PSO1_Baseline
RIN = 8.5
PSO1_W2
RIN = 8.9
PSO1_W4

Figure 2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10
8
6
4
2
0
TSS
Baseline
Week 2
Week 4
b
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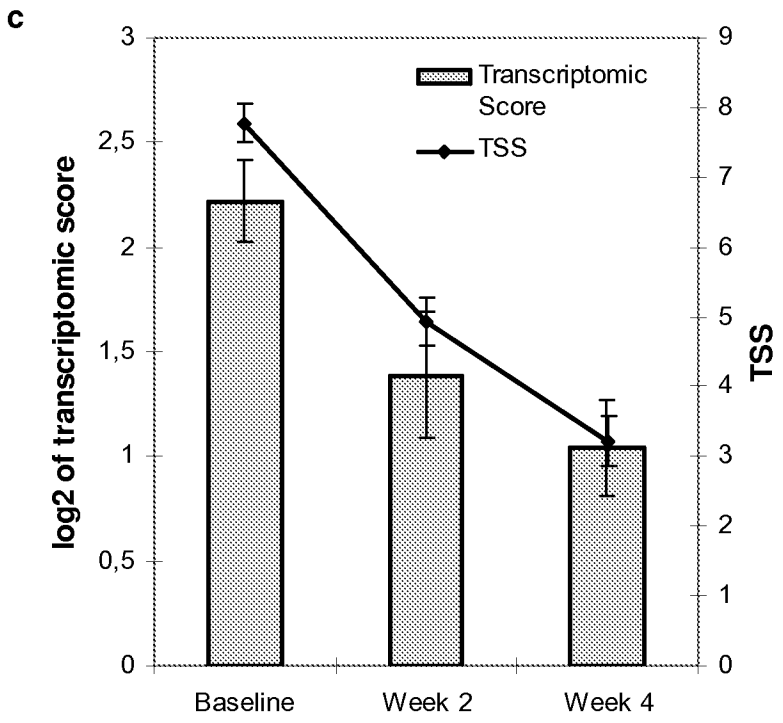
4,00
2,00
0,00
-2,00
-4,00
log 2 of transcriptomic score
Baseline
Week 2
Week 4

Figure 2 C


c
log2 of transcriptomic score
TSS
Transcriptomic Score
TSS
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2,5
2
1,5
1
0,5
0
9
8
7
6
5
4
3
2
1
0
Baseline
Week 2
Week 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/064551

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHOU XIANGHONG ET AL: "Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array." PHYSIOLOGICAL GENOMICS 18 MAR 2003, vol. 13, no. 1, 18 March 2003 (2003-03-18), pages 69-78, XP002512061 ISSN: 1531-2267 cited in the application the whole document	16-19

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2009	05/02/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cornelis, Karen

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	QUEKENBORN-TRINQUET V ET AL: "Gene expression profiles in psoriasis: analysis of impact of body site location and clinical severity" BRITISH JOURNAL OF DERMATOLOGY, vol. 152, no. 3, March 2005 (2005-03), pages 489-504, XP002512062 ISSN: 0007-0963 cited in the application the whole document	16-19
X	WO 2005/100603 A (DERMTECH INTERNAT [US]; BENSON NICHOLAS R [US]) 27 October 2005 (2005-10-27) the whole document	8-12
X	WO 2005/121374 A (OKLAHOMA MED RES FOUND [US]; WELLA AG [DE]; CENTOLA MICHAEL B; THEDERA) 22 December 2005 (2005-12-22) the whole document	1-7,14, 15
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/064551

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		JP 2007531529 T	08-11-2007
		US 2007202540 A1	30-08-2007
		US 2005221334 A1	06-10-2005
WO 2005121374 A	22-12-2005	NONE	
WO 2007076523 A	05-07-2007	AU 2006330410 A1	05-07-2007
		CA 2635690 A1	05-07-2007
		EP 1977007 A2	08-10-2008