



**Évaluation clinique précoce en dermatologie par la médecine translationnelle: application pour un nouvel agent topique dans le traitement du psoriasis = Early screening in dermatology using translational medicine: application for a new topical agent in the treatment of psoriasis**

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UNIVERSITE JOSEPH FOURIER  
FACULTE DE PHARMACIE DE GRENOBLE

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**EVALUATION CLINIQUE PRECOCE EN DERMATOLOGIE PAR LA MEDECINE  
TRANSLATIONNELLE: APPLICATION POUR UN NOUVEL AGENT TOPIQUE DANS  
LE TRAITEMENT DU PSORIASIS**

**EARLY SCREENING IN DERMATOLOGY USING TRANSLATIONAL MEDICINE:  
APPLICATION FOR A NEW TOPICAL AGENT IN THE TREATMENT OF PSORIASIS**

THESE  
PRESENTEE POUR L'OBTENTION DU TITRE DE DOCTEUR EN PHARMACIE  
DIPLOME D'ETAT

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**HP2:** Hypoxie Physiopathologie Respiratoire et Cardiovasculaire

**IAB:** Institut Albert Bonniot, Centre de Recherche « Oncogenèse et Ontogenèse »

**IBS :** Institut de Biologie Structurale

**LAPM:** Laboratoire Adaptation et Pathogénèse des Microorganismes

**LBFA:** Laboratoire de Bioénergétique Fondamentale et Appliquée

**LCBM:** Laboratoire Chimie et Biologie des Métaux

**LCIB:** Laboratoire de Chimie Organique et Biologie

**LECA:** Laboratoire d'Ecologie Alpine

**TIMC-IMAG:** Laboratoire Technique de l'Imagerie, de la Modélisation et de Cognition

**UVHCI:** Unit of Virus Host Cell Interactions



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**UVHCI:** Unit of Virus Host Cell Interactions

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## - FOREWORD -

This Thesis was elaborated during my Master 2 placement period spent in the *Early Clinical Evaluation* (ECE) department of the world first dermatological company called *Galderma*.

Created in 1981, this pharmaceutical company is a joint venture between *Nestlé* and *l'Oréal* employing more than 3 000 collaborators in 31 subsidiaries worldwide. Under the direction of *Humberto C. Antunes* (CEO), the company represents 7.2% of the worldwide dermatological market share in 2009. 20.8% of its turnover (978 Millions) is dedicated to innovation and development of drugs and medical solutions, issued from research. Galderma R&D activities are based in Sophia Antipolis (France), Princeton (USA) and Tokyo (Japan). Committed to the future of dermatology, Galderma focuses on pathologies such as acne, psoriasis, rosacea, atopic dermatitis, pigmentation disorders, skin aging, skin cancer and onychomycosis.

The choice of this thesis was considered according to the thoughts and activities performed during my placement within the early clinical evaluation department. The title of the Thesis is the following: '*Early screening in dermatology using translational medicine: Application for a new topical agent in the treatment of psoriasis*' which is, in my opinion, a good summary of the ECE objectives and activities in which I participated in this company.

Before reaching this thought, I had to understand the company strategy in terms of research and development objectives. I have met a large number of people in early development such as managers of the early clinical department, advanced pharmacology, early ADMET, early formulation, toxicology, etc... These meetings helped me appreciate their mission, their research strategy, as well as their actions and objectives facing concerns of the current approach in early drug development, especially in the treatment of psoriasis. Indeed, there is a strong will to integrate translational medicine at the interface between Research and Development.

My involvement in scientific documentation writing, setting-up, follow-up and data analyses of several clinical studies helped me appreciate the rational and the development strategy involved in clinical projects. In addition, working alongside clinical scientists, researchers and clinical trial administrators made me familiarise with jobs in clinical research as well as the required skills. Besides, interactions with researchers, statisticians, data manager, quality insurance, pharmacovigilance (etc...) showed me how important it is to work in multidisciplinary teams.

## Table of Contents

- ACKNOWLEDGEMENTS - .....	VI
- FOREWORD - .....	VII
- LIST OF FIGURES – .....	XI
- LIST OF ABBREVIATION - .....	XIII
- INTRODUCTION (FRANÇAIS) - .....	XIV
- RESUME DE LA THESE (FRANÇAIS) - .....	XV

<b>CHAPTER 1: BACKGROUND AND LITERATURE REVIEW – .....</b>	<b>1</b>
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INTRODUCTION .....	1
1.1 CURRENT CHALLENGES FACED BY PHARMACEUTICAL INDUSTRY .....	4
1.1.1 PHARMACEUTICAL COMPANIES ARE FACING A RESEARCH & DEVELOPMENT PRODUCTIVITY GAP .....	4
1.1.1.1 DRUG DISCOVERY, DEVELOPMENT AND APPROVAL PROCESS .....	4
1.1.1.2 RESEARCH & DEVELOPMENT EXPENDITURE VERSUS NEW MOLECULAR ENTITIES OUTPUT .....	4
1.1.1.3 ATTRITION RATE .....	6
1.1.1.4 REASONS FOR DEVELOPMENT FAILURES .....	8
1.1.2 CHANGING DEVELOPMENT PARADIGM .....	9
1.1.2.1 LEARN-AND-CONFIRM PARADIGM PROPOSED BY <i>SHEINER</i> .....	9
1.1.2.2 THE FDA'S CRITICAL PATH CHALLENGE .....	10
1.1.2.3 CRITICAL PATH INITIATIVE .....	12
1.2 RATIONALE AND CHALLENGES IN DRUG DISCOVERY AND DEVELOPMENT IN PSORIASIS .....	14
1.2.1 GENERALITIES ON PSORIASIS .....	14
1.2.1.1 EPIDEMIOLOGIC & CLINICAL FEATURES .....	14
1.2.1.2 AETIOLOGY AND PATHOPHYSIOLOGY .....	16
1.2.2 MANAGEMENT OF PSORIASIS DISEASE: A FOCUS ON TOPICAL THERAPIES .....	18
1.2.2.1 PRESENT STATE OF MARKETED TOPICAL THERAPIES IN PSORIASIS .....	18
1.2.2.2 CONSENSUS, GUIDELINES, AND EVIDENCE-BASED DERMATOLOGY: .....	21
1.2.2.3 NEW DIRECTIONS FOR FUTURE TOPICAL THERAPY OF PSORIASIS .....	21
1.3 LIMITATIONS AND PERSPECTIVES IN DEVELOPING NEW TOPICAL THERAPIES FOR PSORIASIS .....	24
1.3.1 PARTICULARITIES AND CONCERNS REGARDING THE ROUTE OF ADMINISTRATION .....	24
1.3.1.1 TYPE OF MOLECULE - SKIN BARRIER & TOPICAL DELIVERY .....	25
1.3.1.2 PERCUTANEOUS ABSORPTION & FORMULATION ASPECTS .....	27
1.3.1.3 TOPICAL APPLICATION ON THE SKIN .....	28
1.3.1.4 SAMPLING METHODS .....	29
1.3.1.5 REGULATORY GUIDELINES .....	29
1.3.1.6 TOPICAL DRUG DEVELOPMENT TIME .....	29
1.3.2 LIMITATIONS OF CURRENT NON-CLINICAL MODELS IN PSORIASIS RESEARCH .....	30
1.3.2.1 PHARMACOLOGICAL MODELS OF PSORIASIS & PERSPECTIVES .....	31
1.3.2.2 DERMAL PHARMACOKINETICS MODELS & PROSPECTS .....	35
1.3.2.3 SAFE FIRST-IN-MAN STARTING DOSE & SYSTEMIC TOXICITY & LOCAL TOLERANCE .....	38
1.4 EARLY CLINICAL TRIALS IN PSORIASIS .....	39
1.4.1 STREAMLINING OF CLINICAL RESEARCH .....	39
1.4.2 INNOVATION IN CLINICAL TRIAL DESIGNS & NOVEL SCREENING METHODOLOGIES IN PSORIASIS .....	40
1.4.2.1 INNOVATION IN CLINICAL TRIAL STRATEGIES .....	41
1.4.2.2 INNOVATIVE CLINICAL TRIAL DESIGNS IN DERMATOLOGY AND PARTICULARLY IN PSORIASIS. ....	43
1.4.2.3 NEW TOOLS TO ASSESS HUMAN PK AND/ OR PD SKIN PARAMETERS .....	46

<b>CHAPTER 2: AIMS &amp; OBJECTIVES –</b>	<b>52</b>
2.1 HYPOTHESIS .....	52
2.2 AIMS.....	53
2.3 OBJECTIVES .....	54
<b>CHAPTER 3: MATERIALS &amp; METHODS –</b>	<b>56</b>
3.1 MATERIALS & METHODS USED FOR THE LITERATURE REVIEW .....	56
3.2 MATERIALS & METHODS USED FOR THE EARLY CLINICAL STUDIES OF INTEREST .....	57
3.2.1 21-DAY TOLERANCE PHASE I HUMAN PHARMACOLOGY IN HEALTHY VOLUNTEERS .....	58
3.2.2 PoC PHASE I HUMAN PHARMACOLOGY IN PSORIATIC PATIENTS .....	60
3.2.3 EVALUATION TOOLS USED FOR DERMAL MEASUREMENTS .....	63
3.3 MATERIALS & METHODS OF THE NON-CLINICAL STUDY OF INTEREST.....	64
3.4 STATISTICAL ANALYSIS OF THE SKIN DISTRIBUTION RESULTS .....	65
<b>CHAPTER 4: RESULTS –</b>	<b>66</b>
4.1 SUMMARY OF THE SKIN DISTRIBUTION RESULTS IN HEALTHY VOLUNTEERS.....	66
4.1.1 INTRODUCTION .....	66
4.1.2 KEY RESULTS.....	66
4.1.3 DETAILED RESULTS.....	67
4.1.3.1 DRUG A CUMULATED CONCENTRATION IN THE SC, THE (E+D) AND IN THE TOTAL SKIN (SC+E+D):	67
4.1.3.2 DRUG A CONCENTRATION PROFILE TO DETERMINE DRUG DISTRIBUTION IN THE SC .....	68
4.2 SUMMARY OF THE SKIN DISTRIBUTION RESULTS IN PSORIATIC PATIENTS.....	69
4.2.1 INTRODUCTION.....	69
4.2.2 KEY RESULTS .....	69
4.2.3 DETAILED RESULTS .....	70
4.2.3.1 DRUG A CUMULATED CONCENTRATION IN THE SC, THE (E+D) AND THE TOTAL SKIN (SC+E+D):	70
4.2.3.2 DRUG A CONCENTRATION PROFILE TO DETERMINE DRUG DISTRIBUTION IN THE SC .....	72
4.3 SUMMARY RESULTS OF <i>IN-VITRO</i> LIBERATION PENETRATION STUDY .....	73
<b>CHAPTER 5: DISCUSSION –</b>	<b>76</b>
5.1 INTRODUCTION .....	76
5.2 CLINICAL SKIN DISTRIBUTION DATA CROSS ANALYSIS: NORMAL SKIN (HEALTHY VOLUNTEERS) <i>VERSUS</i> PSORIATIC SKIN (PATIENTS).....	78
5.2.1 DATA COMPARISON .....	78
5.2.2 DOES PSORIATIC SKIN (I.E. PATHOLOGICAL SKIN) IMPACTS <i>DRUG A</i> PENETRATION? COULD HEALTHY VOLUNTEERS BE PHARMACOLOGICAL MODELS FOR PSORIATIC PATIENTS?.....	81
5.2.3 LIMITS IN THE INTERPRETATION. ....	82
5.2.4 WERE THERE ANY RELATIONS BETWEEN SKIN DISTRIBUTION RESULTS SAFETY AND EFFICACY OBJECTIVES OF THE STUDY PERFORMED IN PSORIATIC PATIENTS .....	83
5.3. OVERALL CROSS ANALYSIS.....	85
5.3.1 DATA COMPARISON.....	85
5.3.2 DOES LIBERATION-PENETRATION STUDY REFLECT CLINICAL SITUATIONS IN HEALTHY VOLUNTEERS AND PSORIATIC PATIENTS TREATED WITH <i>DRUG A</i> ? .....	88
<b>CONCLUSION –</b>	<b>92</b>
- CONCLUSION (FRANÇAIS) - .....	95
- REFERENCES - .....	98

- APPENDICES -.....	103
- SERMENT DES APOTHICAIRES -.....	106
- ABSTRACT -.....	107

**- LIST OF FIGURES -**

Figure n°1	The drug discovery, development and approval specific to the FDA.	4
Figure n°2	R&D expenditures for each new prescription drug between 1979 and 2005.	5
Figure n°3	NMEs and Biologicals approved by the FDA between 1996 and 2008.	5
Figure n°4	Global R & D Expenditure, Development Times, Global pharmaceutical Sales, New Molecular Entity (NME) Output: 1994-2007.	6
Figure n°5	Transition probabilities between phases of drug development of the 50 largest pharmaceutical companies.	7
Figure n°6	Reasons for attrition between 1991 and 2000.	9
Figure n°7	The learn-and-confirm paradigm in drug development.	10
Figure n°8	Research support for product development.	11
Figure n°9	Working in Three Dimensions along the Critical Path.	12
Figure n°10	Chronic plaque psoriasis.	15
Figure n°11	Single plaque of psoriasis, well demarcated and heavily scaled.	15
Figure n°12	Immuno-inflammatory and micro-vascular features of psoriasis.	17
Figure n°13	The march of psoriasis.	18
Figure n°14	Type of therapeutics prescribed to patients with psoriasis depending on disease severity.	19
Figure n°15	<i>a.</i> 2008 sales of selected psoriasis drugs. <i>b.</i> Psoriasis drug pipeline by route of administration.	20
Figure n°16	Structure of human healthy skin and the roles of each separate layer.	24
Figure n°17	Main penetration routes across the non-viable stratum corneum.	26
Figure n°18	Example of what could happen with an inappropriate formulation.	28
Figure n°19	Xenotransplantation of human skin onto SCID mice.	33
Figure n°20	<i>Franz</i> type diffusion cell model using human excised skin.	36
Figure n°21	Decision making throughout drug discovery and development process.	40
Figure n°22	<i>Dumas-Scholtz</i> plaque test on a psoriatic patient's forearm.	43
Figure n°23	<i>a.</i> The most prevalent design of Dermal Microdialysis. <i>b.</i> Dermal Microdialysis performed on a subject.	48
Figure n°24	Skin punch biopsy.	51
Figure n°25	Cutaneous measurements (skin distribution/ concentrations) in different models.	55
Figure n°26	Study schematic.	59
Figure n°27	Study schematic.	61
Figure n°28	Tape stripping: Technical settings	63
Figure n°29	<i>Drug A</i> cumulated concentration in the SC, the (E+D) and in the total skin (SC+E+D), according to <i>Drug A</i> dosage, at Day 19.	67
Figure n°30	<i>Drug A</i> Stratum corneum distribution, according to <i>Drug A</i> dosage, at Day 19.	70
Figure n°31	<i>Drug A</i> accumulated concentration in the SC, (E+D) and the total skin, according to <i>Drug A</i> dosage, at Day 22.	70
Figure n°32	(E+D) <i>Drug A</i> concentration according to <i>Drug A</i> dosage, at Day 22. <i>a.</i> Mean <i>Drug A</i> concentration + error bars; <i>b.</i> Great disparities between individual <i>Drug A</i> levels in the (E+D).	71
Figure n°33	<i>Drug A</i> Stratum corneum distribution, according to <i>Drug A</i> dosage, at Day 22.	72
Figure n°34	<i>a.</i> Individual <i>Drug A</i> 1% SC distribution (patients n° 2, 5 and 12). <i>b.</i> Individual <i>Drug A</i> 5% SC distribution (patients n° 3, 4, 6 and 9)	73



Figure n°35	<i>Drug A</i> accumulated concentration in the Stratum Corneum, the Epidermis, the Dermis and in the Total Skin, according to Drug dosage, at Day 22. <i>a.</i> linear scale; <i>b.</i> logarithmic scale.	74
Figure n°36	Comparison of skin penetration in healthy volunteers <i>versus</i> subjects with psoriasis. <i>a.</i> % of the applied dose that penetrated in the SC. <i>b.</i> % of the applied dose that penetrated in the (E+D).	79
Figure n°37	Ratio between Stratum Corneum and deeper layers <i>Drug A</i> concentration in healthy volunteers and psoriatic patients, depending on <i>Drug A</i> dosage.	79
Figure n°38	<i>a.</i> Histology of psoriasis skin. <i>b.</i> Histology of a normal skin.	81
Figure n°39	The percentage of the penetrated dose recovered in the SC and (E+D) in the three studies, according to <i>Drug A</i> dosage.	86
Figure n°40	<i>a.</i> Mean % of the applied dose that penetrates in the (E + D) layers (Biopsy), at Day22. <i>b.</i> Mean <i>Drug A</i> levels in the (E + D) layers (Biopsy), 24-hours after last applications.	87
Figure n°41	Ratio between SC and (E+D) <i>Drug A</i> concentration in excised human skin, healthy volunteers, psoriatic patients, depending on <i>Drug A</i> dosage.	87

### - LIST OF TABLES -

Table I	Psoriasis: disease severity and prevalence.	15
Table II	Major characteristics of topical treatments in psoriasis in use today.	21
Table III	New potentials in topical therapeutics of psoriasis.	22
Table IV	Existing evaluation models in psoriasis.	31
Table V	Correlation between efficacy of anti-psoriatic drugs in the psoriasis SCID mouse model and clinical experience.	34
Table VI	Summary of the exploratory clinical trials guidelines.	42
Table VII	Definitions of the different type of biomarkers.	45
Table VIII	Different evaluation tools available in psoriasis early clinical development	46
Table IX	Different available methods to assess drug penetration into and/or across the skin.	47
Table X	<i>Drug A</i> skin distribution in the SC, the (E + D) and in the total skin, according to <i>Drug A</i> dosage.	67
Table XI	<i>Drug A</i> skin distribution in the SC, the (E + D) and in the total skin, according to <i>Drug A</i> dosage.	70
Table XII	<i>Drug A</i> skin distribution in the SC, (E + D) and the total skin, according to <i>Drug A</i> dosage.	73
Table XIII	<i>a.</i> Mean skin concentration in tape-strips, biopsies and total skin of three different models: ex-vivo human skin, healthy skin and pathological skin. <i>b.</i> Percentage of the applied dose that penetrates in the different layers of the skin for each model.	76
Table XIV	Main differences in the design of the three studies.	78

### - LIST OF APPENCICES -

Appendix A	Mean concentration and Standard Deviation values of tape-strips, total SC, biopsies (E+D) and Total Skin in healthy volunteers, according to <i>Drug A</i> dosage.	103
Appendix B	Mean concentration, Standard Deviation and Coefficient of Variation values of tape-strips, total SC, (E+D) and Total Skin in psoriatic patients, according to <i>Drug A</i> dosage.	103
Appendix C	Individual data source of <i>Drug A</i> 1% tape-strips, total SC, (E+D) and Total Skin in psoriatic patients.	104
Appendix D	Individual data source of <i>Drug A</i> 5% tape-strips, total SC, (E+D) and the Total Skin, in psoriatic patients.	104
Appendix E	Ratio of the mean concentration in the Stratum Corneum and the Epidermis + Dermis in the three models, according to <i>Drug A</i> dosage.	105
Appendix F	Skin distribution: Percentage of the penetrated dose recovered in the SC and E+D in the three models, according to <i>Drug A</i> dosage.	105

## - LIST OF ABBREVIATION -

ADME	Absorption – Distribution – Metabolisation – Elimination
BLA	Biologic License Application
BSA	Body Surface Area
CTs	Clinical Trials
CTD	Common Technical Document
CUTIS	Clinical Unit for Testing and Investigation of Skin
CV	Coefficient of Variability
D	Dermis
DPK	Dermatopharmacokinetic
DS	Dumas-Scholtz
E	Epidermis
ECE	Early Clinical development
eIND	Exploratory Investigational New Drug
EMA	European Medicines Agency
EMEA	European Medicinal Evaluation Agency (= European Medicines Agency)
eTrials	Exploratory Clinical Trials
FDA	Food and Drug Administration
FIM	First-In-Man
GCs	Glucocorticoids
HED	Human Equivalent Dose
HPLC	High Performance Liquid Chromatography
HVs	Healthy Volunteers
ICH	International Conference of Harmonisation
IL	Interleukine
IMPD	Investigational Medicinal Product Dossier
IND	Investigational New Drug
LC-MS/MS	Liquid Chromatography-Mass Spectrometry/Mass spectrometry
MAPK	Mitogen-Activated Protein Kinases
NCE	New Chemical Entities
NDA	New Drug Application
NME	New Molecular Entities
NOAEL	No Observable Adverse Effect Level
OFM	Open Flow Microdialysis
PD	Pharmacodynamic
PDE4	Phosphodiesterase 4
PK	Pharmacokinetic
PoC	Proof of Concept
PoE	Proof of Efficacy
R & D	Research and Development
SC	Stratum Corneum
SCID	Severe Combined ImmunoDeficiency
SD	Standard Deviation
SICI	Scientific Information & Competitive Intelligence
TD	Toxicodynamic
TLRs	Toll-Like Receptors
TNF	Tumor Necrosis Factor
TSS	Total Sum Score
VEGF	Vascular Endothelial Growth Factor
VRD	Vitamin D Receptor

## - INTRODUCTION (français) -

Le psoriasis est une pathologie chronique inflammatoire caractérisée par une hyperplasie et une différenciation incomplète des kératinocytes. Elle touche entre 0,6 et 4,8% de la population générale. La recherche sur cette maladie dermatologique est abondante, avec plus 29 000 articles publiés dans *PubMed* à ce jour. Cependant, il reste encore un certain nombre de questions en suspens. Alors que la recherche biomédicale est en pleine croissance, il y a un écart grandissant entre les découvertes faites en recherche et la mise en application au lit du patient. C'est alors que l'évaluation clinique précoce, à l'interface entre la recherche et le développement clinique, utilise une approche par la Médecine Translationnelle, de part ses designs cliniques innovants et le transfert d'informations constant avec les services de recherche.

Les critères à prendre en compte, lors de la recherche et du développement de nouvelles entités topiques pour le psoriasis, ainsi qu'une réflexion sur l'implémentation de la médecine translationnelle en dermatologie, sont abordés dans la partie bibliographie (*chapitre 1*).

La seconde partie de cette thèse illustre la mise en application d'une approche translationnelle lors du développement clinique précoce d'un agent topique dans le traitement du psoriasis. Elle consiste à réaliser une analyse transversale qui permettra d'évaluer (1) l'impact de la pathologie sur la pénétration d'une *molécule A* dans les différentes couches de la peau ; (2) la capacité d'une étude *in-vitro* de libération-pénétration à prédire le profil de pénétration chez l'homme. Les objectifs de cette analyse sont présentés dans le *chapitre 2*, les modèles et les protocoles d'étude en *chapitre 3*. Les résultats seront décrits dans le *chapitre 4* puis comparés et discutés dans le *chapitre 5*. La conclusion permettra notamment de montrer l'impact des résultats de pénétration de la *molécule A* sur le plan de développement du *produit A* (*chapitre 6*).

## - RESUME DE LA THESE (français) -

Cette section résume les prérequis et les points essentiels de la thèse. Le psoriasis et ses traitements (principalement topiques) seront décrits dans une première partie, suivis de la bibliographie et de l'introduction de l'analyse transversale. Enfin, les résultats importants de l'analyse seront exposés et discutés.

### 1. La pathologie

Le psoriasis est une pathologie chronique inflammatoire avec une composante génétique touchant entre 0,6 et 4,8% de la population générale. La forme clinique la plus fréquente, appelée *Psoriasis Vulgaire* ou psoriasis en plaques, représente environ 85 à 90% des cas. Cette maladie dermatologique se caractérise par une hyperplasie et une maturation incomplète des keratinocytes, entraînant la formation de plaques squameuses, principalement au niveau des zones bastions (coudes, genoux), des lombes et/ou du cuir chevelu. La sévérité dépend de la surface du corps atteinte par la maladie (*Body Surface Area*) ; la plupart des patients (65 à 80%) sont atteints de psoriasis peu étendu (< 3% de la surface du corps atteinte).

Cette maladie se manifeste généralement par poussées. Son étiologie est à la fois génétique et environnementale. En effet, un tiers des patients atteints de psoriasis vulgaire ont un proche de premier degré également atteint. Les facteurs environnementaux peuvent être infectieux (tel que le streptocoque, le virus de l'immunodéficience humaine), médicamenteux (dont les bêtabloquants, le lithium et les antipaludéens), ou liés au stress, à un traumatisme ou au tabagisme.

Bien que cette maladie ne soit pour l'instant pas curable, il existe un large choix de traitements, prescrits en fonction de la sévérité de la pathologie. Par exemple, un psoriasis peu étendu sera traité par médication topique telle que les dermocorticoïdes (propionate de clobétasol), les dérivés de la vitamines D (calcipotriol, calcitriol) ou en association (calcipotriol + bethaméthasone), les rétinoïdes (tazarotène). Il sera traité en association avec un autre type de traitement pour les formes plus sévères : photothérapies (UVB, PUVA thérapie), traitement par voie orale (acitrétine) ou biothérapies pour les formes les plus sévères (efalizumab, etanercept, adalimumab, infliximab). Cependant ces traitements ne sont pas toujours satisfaisants de part leur

manque d'efficacité et/ou leur mauvaise tolérance. C'est pourquoi le psoriasis reste l'un des principaux axes de recherche en dermatologie.

Le psoriasis pourrait être classé comme maladie auto-immune caractérisée par une inflammation chronique en l'absence d'agents infectieux ou antigènes connus. La physiopathologie du psoriasis n'est pas encore entièrement expliquée. On sait tout de même que le psoriasis présente des caractéristiques immuno-inflammatoires mais aussi micro-vasculaires. En effet, des infiltrats leucocytaires et cytokines pro-inflammatoires mais aussi une augmentation du facteur de croissance vasculaire endothéliale (VEGF) sont présents au niveau des zones atteintes. Ce dernier entraîne une augmentation et une dilatation des vaisseaux facilitant, entre autre, la migration des leucocytes au niveau des lésions.

Alors que l'indication dans le psoriasis des traitements topiques énumérés précédemment a été découverte de façon empirique pour la plupart, une stratégie plus pragmatique permettrait de répondre aux besoins. Pour cela, une meilleure connaissance de la maladie (pharmacogénétique, recherche de biomarqueurs) permettrait développer des traitements agissant directement sur la physiopathologie de la maladie. Une telle approche serait d'interférer avec les voies métaboliques impliquées dans la production de cytokines pro-inflammatoires du psoriasis (exemples: les inhibiteurs de kinases : inhibiteur JAK, p38-MAPK ou inhibiteurs de phosphodiesterases : PDE4, PDE7).

## 2. Bibliographie

Alors que la recherche biomédicale est en pleine croissance, il y a un écart grandissant entre les découvertes faites en recherche et la mise en application au lit du patient. Deux principales questions sont abordées dans la partie bibliographie (*cf chapitre 1*) :

- Quels sont les critères à prendre en considération lors de la recherche et du développement de nouvelles entités topiques dans le traitement du psoriasis?
- Comment une approche par la Médecine Translationnelle peut-elle être implémentée en dermatologie dans l'objectif d'améliorer l'évaluation de nouvelles molécules candidates en topique pour traiter le psoriasis ?

Un certain nombre de critères sont à prendre en considération lors de la recherche et du développement de nouvelles entités topiques dans le traitement du psoriasis :

- a. Critères reliés à la pathologie: complexité de la maladie, pertinence des modèles non-cliniques ainsi que les *designs* cliniques utilisés pour évaluer une nouvelle molécule en développement (exemple : design *Dumas-Scholtz* modifié).
- b. Critères non reliés à la pathologie : la voie d'administration (caractéristiques de la peau, de la molécule d'intérêt), l'adéquation des outils d'évaluation utilisés en dermatologie (par exemple, l'exposition systémique ne reflèterait pas l'exposition de la molécule au site d'action).

Une approche par la médecine translationnelle, également appelée médecine expérimentale, est encouragée par les autorités compétentes et soutenue par la recherche académique. Elle cherche à faire évoluer les pratiques cliniques grâce au transfert de découvertes fondamentales faites en recherche vers le développement clinique (et vice versa). A l'interface entre recherche et développement clinique, la médecine translationnelle peut contribuer à améliorer l'évaluation précoce de nouveaux traitements topiques dans le psoriasis. Pour arriver à cela, les principaux objectifs sont :

- a. Acquérir une meilleure compréhension de la physiopathologie de la maladie ainsi que des prédispositions génétiques associées à cette pathologie. Par exemple, l'identification de polymorphismes suggérant une susceptibilité pour la maladie ou une réponse à un traitement permettrait une prise en charge plus personnalisée : c'est à dire, administrer le bon traitement, au bon patient (jugé répondeur selon son polymorphisme), au bon dosage et au bon moment.
- b. Obtenir des résultats, en recherche non-clinique, plus robustes et plus prédictifs de l'effet et du devenir d'une molécule chez l'homme:
  - Développer de nouveaux modèles non-cliniques ou améliorer les modèles existants en les comparant aux données cliniques, afin qu'ils reflètent au mieux les conditions cliniques (corrélation *in-vitro*, *ex-vivo*, *in-vivo*).

- Valider des critères communs d'évaluation (+ méthodes associées) évaluables *in-vitro* et chez l'homme (exemple : mesure de l'exposition de la molécule d'intérêt au niveau du site d'action *in-vitro* → prédiction de l'exposition chez l'homme ?).
  - Développer des stratégies et méthodologies non-cliniques appropriées afin d'obtenir des informations pertinentes soutenant les décisions de « Go/NoGo » le plus tôt possible (validation de méthodes, études exploratoires).
- c. Optimiser les designs en développement clinique précoce afin d'intégrer une approche translationnelle:
- Réaliser des essais cliniques exploratoires (eTrials) et des études « Preuve-de-Concept » ou « Preuve d'efficacité » avant d'entreprendre les études de Phase I réglementaires.
  - Utiliser des designs adressant le maximum de questions critiques le plus tôt possible ; telles que des données de pharmacocinétique au niveau du site d'action (biopsies de la peau, microdialyse), pharmacodynamie et toxicodynamie (identification de biomarqueurs d'activités pharmacologiques, toxicologiques), des profils de PK/PD.
  - Valider ces techniques d'évaluation en utilisant des produits de référence dont l'efficacité et la tolérance sont connus dans la pathologie ciblée.
  - Identifier et valider des biomarqueurs liés à la maladie (exemple : cytokines pro-inflammatoires dans le psoriasis), et/ou à l'action pharmacologique du médicament (exemple : diminution du TNF $\alpha$  suite à un traitement anti-TNF $\alpha$ ).

Tous ces points sont abordés plus en détails dans le chapitre « *Background and Literature Review* » de cette thèse. Ils permettent d'introduire et surtout de comprendre les objectifs de l'analyse des résultats d'exposition de la *molécule A* dans la peau, dans trois modèles différents. En effet, il est important de récolter, lors de la recherche non-clinique et du développement clinique précoce, un maximum d'informations pertinentes, et ce, afin d'être capable de sélectionner une molécule

prometteuse ou, à l'inverse, d'arrêter le développement d'un candidat médicament non prometteur.

### 3. Mise en application d'une approche translationnelle en développement clinique précoce

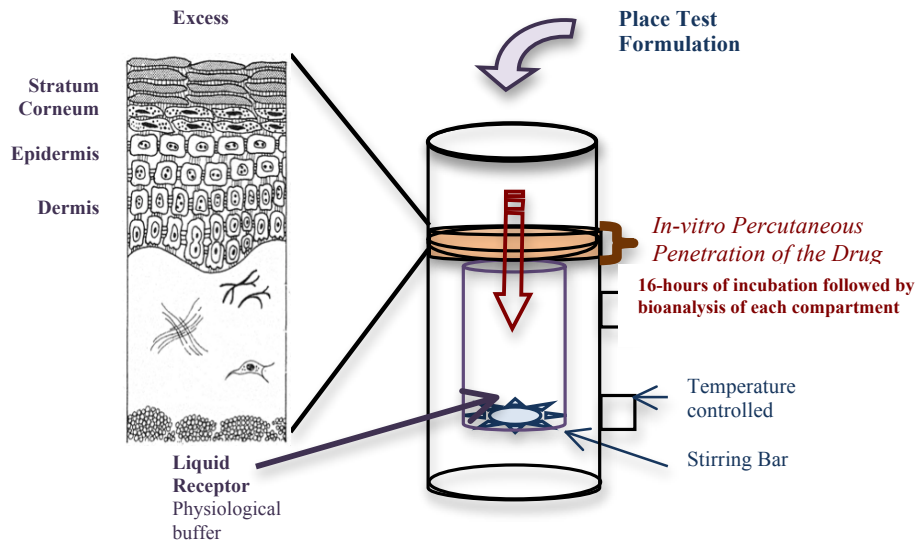
La seconde partie de cette thèse illustre la mise en application d'une approche translationnelle lors du développement clinique précoce d'un agent topique dans le traitement du psoriasis. Elle consiste à réaliser une analyse transversale en comparant les profils de pénétration d'une *molécule A*, après application topique, dans les différents compartiments de la peau (couche cornée / épiderme / derme), dans différents modèles : un modèle non-clinique (étude *in-vitro*) et deux modèles cliniques (études chez le volontaire sain et le patient atteint de psoriasis).

La *molécule A* vient d'un médicament *per os* indiqué dans le traitement d'une maladie non-dermatologique. Ses propriétés pharmacologiques ont été évaluées comme potentiellement utiles pour le traitement du psoriasis en plaque. Ainsi, cette molécule a été reformulée sous forme de crème, pour application topique.

Une description concise des études et de leurs modèles, comparés dans l'analyse transversale, est présentée ci-dessous :

**Etude non-clinique** : Lors du développement du *produit A*, une étude *in-vitro* appelée : libération-pénétration, ou encore étude en cellule de diffusion, ou cellule de *Franz*, a été réalisée. L'objectif était de comparer les différents profils de pénétration de la *molécule A*, au travers de peau humaine non-viable (peau excisée post-chirurgicale), aux concentrations 1%, 3% et 5%. Il y avait trois donneurs et deux cellules/ donneurs. La figure ci-dessous illustre le modèle et la technique de cette étude (ce modèle est détaillé en *section 1.3.2.2*). Chaque cellule de diffusion fait 2cm<sup>2</sup>. Ce modèle est validé pour une application unique suivie d'environ 16-heures d'incubation avant la bioanalyse. Les compartiments suivants ont été analysés : l'excès de formulation (produit non absorbé), la couche cornée, l'épiderme, le derme ainsi que le liquide récepteur (concentration absorbée au delà du derme, pouvant atteindre la circulation sanguine). Seules les données de la couche cornée, de l'épiderme + derme et de la peau totale (couche cornée + épiderme + derme) nous intéressent pour l'analyse.





Cellule de Franz ou cellule de diffusion utilisée en étude *in-vitro* de libération/pénétration.

### Etudes cliniques :

Les deux études cliniques étaient des études de phase I, mono-centriques, randomisées, « investigateur masqué » (c'est-à-dire ne sachant pas quels produits/concentrations étaient appliqués sur les différentes mini-zones traitées), en comparaison intra-individuelle (chaque sujet recevant tous les produits/concentrations) et en doses répétées. Il n'y avait pas de bénéfice thérapeutique direct pour les sujets.

- *Etude chez les volontaires sains* : 27 sujets masculins ont reçu une application par jour (5jours/semaine, pendant 3 semaines) du *produit A* aux concentrations 1%, 3% et 5% (+ véhicule + comparateurs positifs) sur des mini-zones (environ 3cm<sup>2</sup>) dans le dos. Les objectifs principaux de l'étude étaient l'évaluation du potentiel d'irritation cumulée du *produit A* aux concentrations 1%, 3% et 5%, ainsi que la tolérance systémique. L'objectif exploratoire était la quantification de la *molécule A* au niveau de la peau sur l'une des zones traitées par le *produit A*, 24-heures après la dernière application des produits (à Jour19, après 14 applications au total).
- *Etude chez les patients atteints de psoriasis*: 22 sujets (masculins ou féminins) atteints de « psoriasis vulgaire » stable en plaques, ont reçu une application par jour (6jours/semaine, pendant 3 semaines) du *produit A* aux concentrations 1%, 2% 3% et 5% (+ véhicule + comparateurs positifs) sur des mini-zones (environ 3cm<sup>2</sup>) situées sur des plaques de psoriasis sélectionnées par l'investigateur (tronc, bras, jambes). Les objectifs principaux de l'étude étaient l'évaluation de la tolérance du

*produit A* au niveau local et systémique, ainsi qu'une évaluation clinique de l'efficacité de la molécule, par rapport à son véhicule et aux comparateurs positifs. L'objectif exploratoire était la quantification de la *molécule A* au niveau de la peau, 24-heures après la dernière application des produits (à Jour 22, après 18 applications au total).

Chaque étude a été réalisée séparément, mais l'objectif exploratoire était à chaque fois d'évaluer la concentration de la *molécule A* au niveau de la couche cornée grâce à la technique de « tape stripping » et au niveau de l'épiderme et du derme (là où se situe le site d'action) après une biopsie d'environ 3mm de diamètre réalisée après le tape stripping.

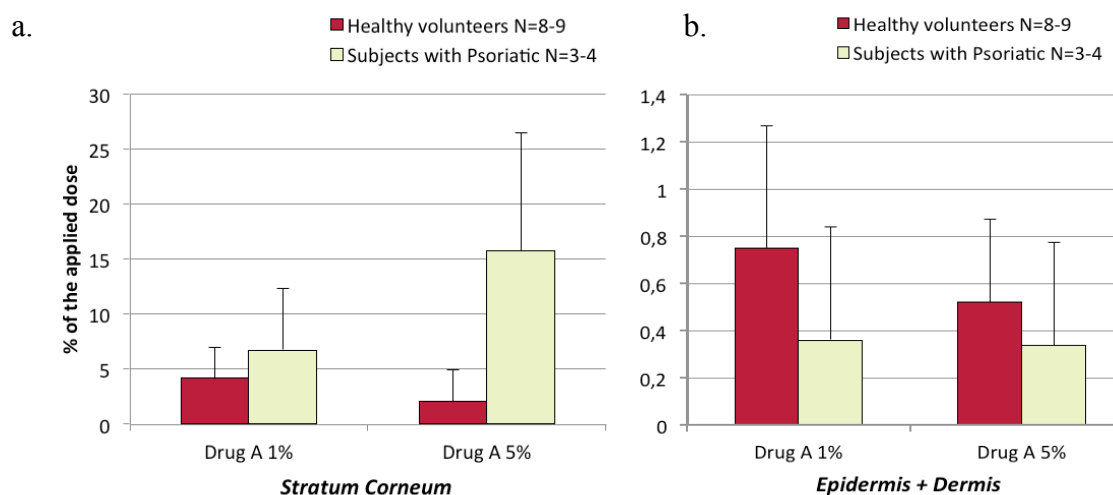
L'objectif principal de l'analyse transversale était de tenter de répondre aux deux questions suivantes :

- a. Quel est l'impact de la pathologie psoriasis sur la pénétration de la *molécule A* dans les différentes couches de la peau ? Pour cela, une comparaison des résultats volontaires sains *versus* patients atteints de psoriasis a été réalisée.
- b. L'étude de libération-pénétration réalisée *in-vitro* sur des peaux humaines excisées est-elle capable de prédire si la molécule d'intérêt a atteint son site d'action chez le patient atteint de psoriasis, et à quelle concentration ? Le profil de pénétration de la *molécule A* dans la peau est-il le même *in-vitro* et *in-vivo* ?

Avant toute analyse de résultats, il faut noter que les protocoles étaient très différents d'une étude à l'autre car les objectifs principaux étaient différents pour chaque étude. Les principales différences se retrouvent au niveau des quantités de *produit A* appliquées (50µL chez sur sujet psoriasique, 10µL chez le volontaire sain et 4µL par cellule de *Franz in-vitro*), de la fréquence d'application (applications répétées en clinique *versus* application unique en non-clinique), du nombre d'échantillons (6 échantillons *in-vitro*, 26 dans l'étude volontaires sains et 7 dans l'étude psoriasis). C'est pour cette raison que la plupart des résultats analysés sont en pourcentage de dose appliquée pénétrant dans la peau, et non en valeurs absolues (concentrations).

#### **a. Impact de la pathologie sur la pénétration de la *molécule A***

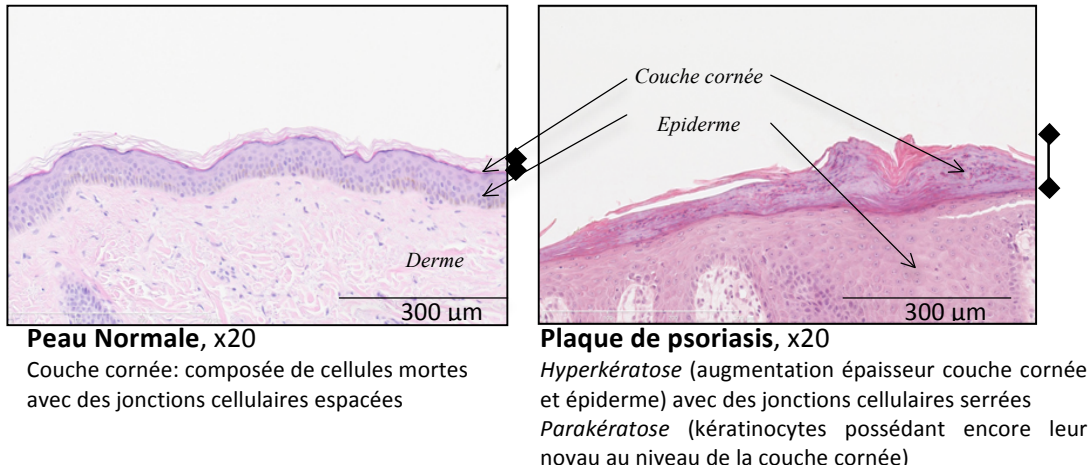
Ce graphique présente les moyennes des pourcentages de doses appliquées qui pénètrent au niveau de la couche cornée (SC ; *Figure a.*) et de l'épiderme + derme (E+D ; *Figure b.*), sur peau normale (volontaires sains) et peau psoriasique (patients).



Comparaison de la pénétration de la *molécule A* dans la peau (en terme de pourcentage de la dose appliquée) chez le volontaire sain *versus* sujet atteint de psoriasis. *Stratum corneum* = Couche cornée ; *Epidermis+Dermis* = Epiderme + Derme.

On remarque que le pourcentage de *molécule A* pénétrée est plus important au niveau SC qu'au niveau (E+D), dans les 2 modèles. Par exemple, pour la *molécule A* 5% chez le patient: 15,74% de pénétration au niveau de la couche cornée contre 0,34% de pénétration jusqu'au site d'action (E+D). Une dose proportionnalité a été statistiquement observée au niveau de l'(E+D) pour les deux modèles (*cf Figure 40b chapitre 5*). Il ne semble cependant pas y avoir de dose proportionnalité au niveau de la couche cornée (tests statistiques non réalisés).

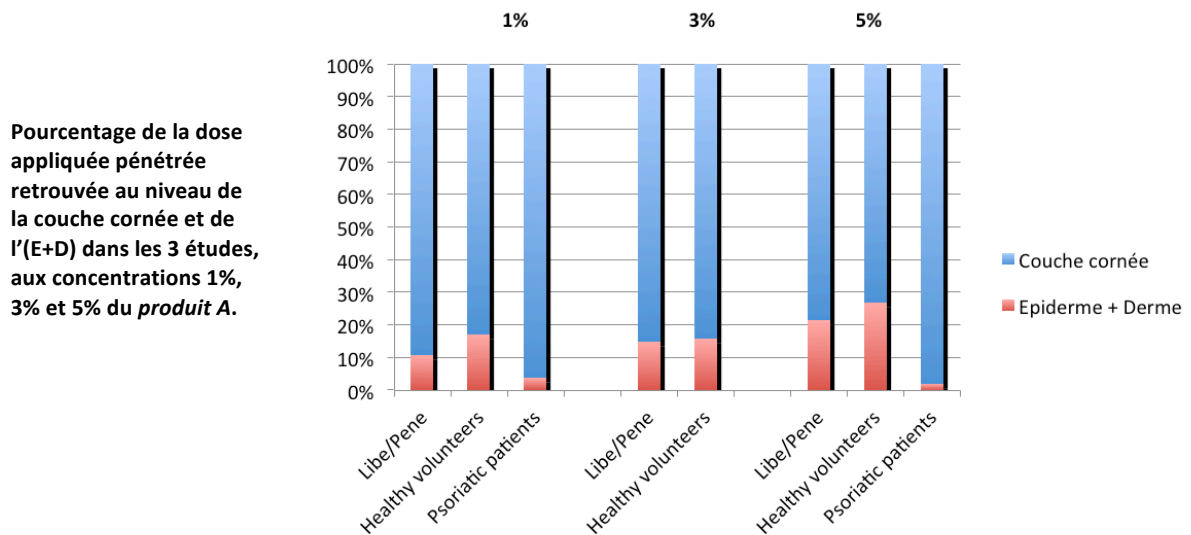
L'impact de la pathologie sur la pénétration de la *molécule A* à travers la peau semble évident. Ceci s'explique principalement par les différences histologiques entre une peau psoriasique et une peau normale. En effet, sa couche cornée plus épaisse que celle d'une peau normale semble lui conférer un effet réservoir. De plus, les cellules (keratinocytes possédant encore leur noyau) en jonctions serrées au niveau de la couche cornée d'une plaque de psoriasis, en opposition aux cellules mortes (keratinocytes sans noyau) plus espacées de la peau normale, rendent la pénétration de molécules au travers de la barrière de la couche cornée plus difficile. Comme suggérée par les résultats, la pénétration jusqu'au site d'action semble être diminuée sur une peau psoriasique par rapport à celle d'une peau normale.



On peut tout de même noter que l'analyse est limitée par un certains nombre de paramètres, tels que: les quantités appliquées très différentes, le nombre d'échantillons faible pour l'étude psoriasis, les variabilités inter & intra-individuelles (localisation des zones d'application, sévérité des plaques de psoriasis) mais aussi la procédure d'échantillonnage (particulièrement pour le tape stripping). Ceci se caractérise notamment par des barres d'erreurs non négligeables.

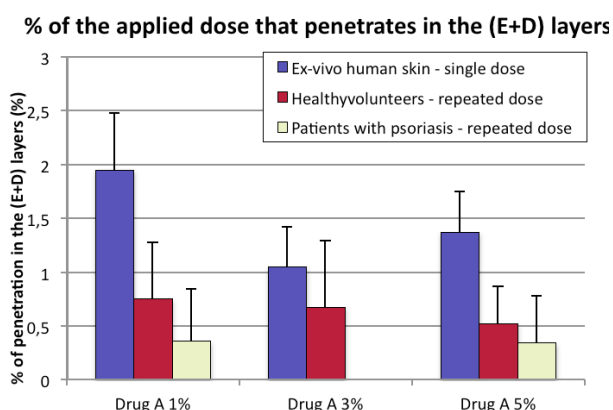
#### b. Comparaison des résultats de l'étude non-clinique *versus* études cliniques

Le graphique suivant présente le pourcentage de la dose pénétrée retrouvée au niveau de la couche cornée et de l'(E+D) pour les concentrations 1%, 3% (sauf pour les patients atteints de psoriasis) et 5%, dans chaque modèle. On peut observer que la distribution de la *molécule A* est proche entre l'étude *in-vitro* et le volontaire sain avec, respectivement, 78% et 73% de *molécule A* 5% retrouvée au niveau de la couche cornée. Cependant, chez le patient psoriasique, 98% de la *molécule A* 5% est retrouvée au niveau de la couche cornée. Son profil de distribution est donc éloigné de celui du volontaire sain et de la peau excisée *in-vitro*.

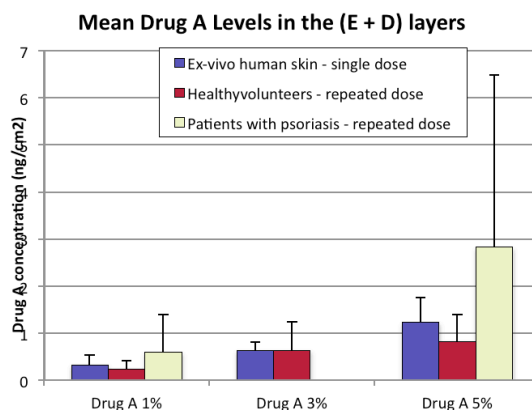


Les graphiques suivants représentent les pourcentages moyens de doses appliquées qui pénètrent (*Figure a*) et les concentrations moyennes accumulées (*Figure b*) au niveau de l'épiderme + derme (E+D), pour chacun des modèles.

a.



b.



**a.** Pourcentage moyen de la dose appliquée qui pénètre jusqu'à l'(E + D). **b.** Concentration moyenne de *molécule A* au niveau de l'(E + D), 24-heures après la dernière application en clinique et 16-heures après l'application en non-clinique.

Bien que les pourcentages de doses appliquées et les concentrations absolues trouvées *in-vitro* et *in-vivo* soient dans la même fourchette, il n'est pas possible d'établir de conclusion définitive sur la capacité de l'étude *in-vitro* à refléter l'homme pour les raisons expliquées précédemment. On peut tout de même dire que la *molécule A* pénètre plus *in-vitro* que *in-vivo*. Il est à noter que cette observation n'est valable que pour cette *molécule A*, avec cette formulation. Le profil de pénétration pourrait être très différent pour une autre molécule et une autre formulation. De plus, un modèle de peau non-viable est forcément limité dans sa capacité à refléter un organe viable tel que la peau, notamment en terme de métabolisation par la peau, de différences en terme de vascularisation etc. Enfin, il est difficile d'harmoniser les protocoles d'études qui permettraient de comparer ces résultats de façon pertinente. La réalisation d'études dédiées à l'évaluation de la pénétration d'une molécule candidate permettrait d'être dans de meilleures conditions pour faire ce genre d'analyse.

## **Chapter 1: BACKGROUND AND LITERATURE REVIEW –**

### **INTRODUCTION**

Psoriasis is a common, chronic inflammatory skin disorder that affects 0.6 to 4.8% of the general population (Naldi, L., 2004). Research into this dermatological pathology has been very popular, leading to more than 29 800 papers published until today (PubMed, 2010), with an ever-increasing rise (Schon, M.P., 2008). However, unanswered questions still remain. At a time when basic biomedical knowledge is increasing exponentially, the gap between bench discovery and bedside application appears to be expanding (FDA, 2004). The questions that will be addressed in the following literature review are:

*1. What criteria are to be taken into consideration for topical anti-psoriasis New Chemical Entities (NCE) discovery & development success?*

*2. How a Translational Medicine approach could lead to improved early screening of new topical treatments for psoriasis?*

1. The criteria that should be taken into consideration include those that are:

- a. Related to the disease: including the complexity of pathophysiology of psoriasis, the relevance of non-clinical models, as well as the relevance of clinical designs used to assess the value of new compounds developed to treat psoriasis, compared to gold standard therapeutics.
- b. But also those not related to the disease:
  - General criteria: such as the current challenges faced by pharmaceutical industries
  - Criteria specific to dermatology, including the route of administration as well as the suitability of the tools available for dermal evaluations.

2. Sponsors, with the help of Academics and the endorsement of Regulatory Authorities are introducing *Translational Medicine* (also called *Experimental Medicine*) at the interface between drug discovery and development. This emerging view of medical practice, as a progression from *Evidence-Based Medicine*, should contribute to improve the process of new drug development, and to reduce late failures' rates.

By translating laboratory-based research into early clinical development and by bringing clinical observations to the laboratory, Translational Medicine may *contribute to the improvement of the early screening of new topical treatments in psoriasis*. The following three objectives will be tackled in the core of the literature review chapter:

- a. To acquire a better understanding of the complex pathophysiology of psoriasis, at a molecular level, including population susceptibility to the disease, leading to research pathogenesis-based treatment and potentially personalised medicine (i.e. prescribing the right treatment, for the right patient, at the right dosage and at the right time).
- b. To obtain more predictable results from pre-clinical research to the clinic by:
  - Developing new and refining existent non-clinical models (i.e. *in-vitro*, *ex-vivo*, *in-vivo* models) and assessing them by comparing their results with those from clinical trials
  - Validating new non-clinical screening methodology by assessing their ability to predict the clinic (e.g. for topical products, comparison of skin penetration data provided by *in-vitro* liberation-penetration tests, *versus* skin distribution data obtained in humans).
  - In addition to regulatory pre-requisites, developing relevant pre-clinical methodologies, considering what information is really needed to be confident for the Go/ No-Go decision before starting clinical development (e.g. exploratory testing, validation of the methods).
- c. To integrate translational medicine in Early Clinical Development in psoriasis by:

- Conducting exploratory trials (eTrials) and Proof-of-Concept (PoC)/ Proof-of-Efficacy (PoE) studies before the traditional phase I package.
- Using new clinical designs addressing several questions earlier in the development such as pharmacokinetic (PK) profiles (e.g. drug bioavailability at the target site thanks to new evaluation tools: skin biopsies, microdialysis...), Pharmacodynamic/ Toxicodynamic information (by the identification of biomarkers early in the development), PK/ PD profile (since both are closely related).
- Validating new clinical screening methodology by using well-established drugs in the concerned disease
- Identifying and validating disease-related (e.g. pro-inflammatory cytokines in psoriasis) or target-related (e.g. diminution of TNF $\alpha$  with an anti-TNF $\alpha$  compound) biomarkers, *in-vivo* and *in-vitro* models (e.g. correlation of the biomarker response between the biopsy data from human psoriatic plaques and those from grafted mice biopsies).

A concrete experimental medicine application will be presented in the second part of this thesis. It consisted in performing a cross analysis that compares the skin penetration profile of a *molecule A*, after topical application, in three different models. Three separate studies were conducted: one *in-vitro* liberation-penetration study (using excised human skin) and two clinical studies performed on healthy volunteers and psoriatic patients. The first aim was to evaluate the impact of the psoriasis pathology on skin penetration by comparing psoriatic skin and healthy skin results. The second aim was to evaluate whether the *in-vitro* study performed with this new drug reflected human skin penetration profile.

Indeed, collecting relevant information from non-clinical studies and from early clinical proof-of-concept studies of new topical agents is important to enable early screening but also enable early discontinuation of the development if the investigational compound is not promising.



## 1.1 Current challenges faced by pharmaceutical industry

### 1.1.1 Pharmaceutical companies are facing a Research & Development productivity gap

#### 1.1.1.1 Drug discovery, development and approval process

Pharmaceutical drug discovery and development is a complex, long, expensive and a high-risk enterprise (Dingemans, J. & Appel-Dingemans, S., 2007). This multidisciplinary process spans many years from the early discovery phase to the approval by regulatory authorities. Typically, it takes as much as 10 to 15 years for an experimental drug to travel from the lab to patients' bedside, according to the *Tufts Center for the Study of Drug Development*. Only five in 5,000 compounds that enter preclinical testing make it to human testing. One in five drugs tested in people is actually approved. (PhRMA, 2010). *Figure 1* presents a simplified overview of the current drug discovery, development and approval process, specific to the Food and Drug Administration (FDA).

Discovery/ Preclinical Testing		Clinical Trials			FDA	Phase IV
Years	6.5	Phase I	Phase II	Phase III		
Test Population	Laboratory and animal studies	20 to 100 healthy volunteers	100 to 500 patient volunteers	1,000 to 5,000 patient volunteers	Review process/ approval	Additional post-marketing testing required by FDA
Purpose	Assess safety, biological activity and formulations	Determine safety and dosage	Evaluate effectiveness, look for side effects	Confirm effectiveness, monitor adverse reactions from long-term use		
Success Rate	5,000 compounds evaluated	5 enter trials			1 approved	

Figure n°1 | **The drug discovery, development and approval process specific to the FDA.** The process is similar in Europe: the IMPD is submitted to the national Competent Authority before any clinical trial; the CTD is submitted for marketing Approval. (PhRMA, 2009)

#### 1.1.1.2 Research & Development expenditure versus new molecular entities output

Indeed, the discovery and development of safe and effective new medicines is a lengthy, increasingly complex and resources intensive process. Pharmaceutical and biotechnology firms invested an estimated \$65.3 billion in Research and Development in 2009, which represents 16.0 % of total sales (PhRMA, 2010). On average, it costs a

company \$1.3 billion investment per successful New Molecular Entity (NME), (PhRMA, 2010) of which only two in ten new drugs, on average, generate sufficient revenues to sustain Research & Development (R&D) or exceed R&D costs (Vernon, J.A. & al., 2009). *Figure 2* shows the increase of development costs between 1979 and 2005.

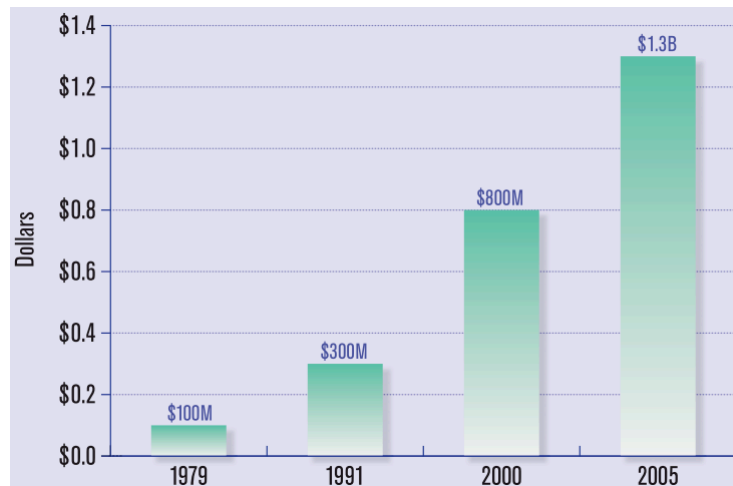


Figure n°2 | **R&D expenditures for each new prescription drug averaged between 1979 and 2005.** (DiMasi, J.A. & Grabowski, H.G., 2007; DiMasi, J.A. & al., 2003).

Despite rising investment in pharmaceutical R&D in the past 30 years, successful development of novel drugs is slowing down. Indeed, the number of new molecular entities (NMEs) (New Chemical Entities (NCEs) and biologicals) submitted to the FDA and the European Medicines Agency (EMA) has stagnated or even declined over the last 15 years (*Figure 3*). In 2002, only 17 NCEs were approved by the FDA (Kola, I. & Landis, J., 2004).

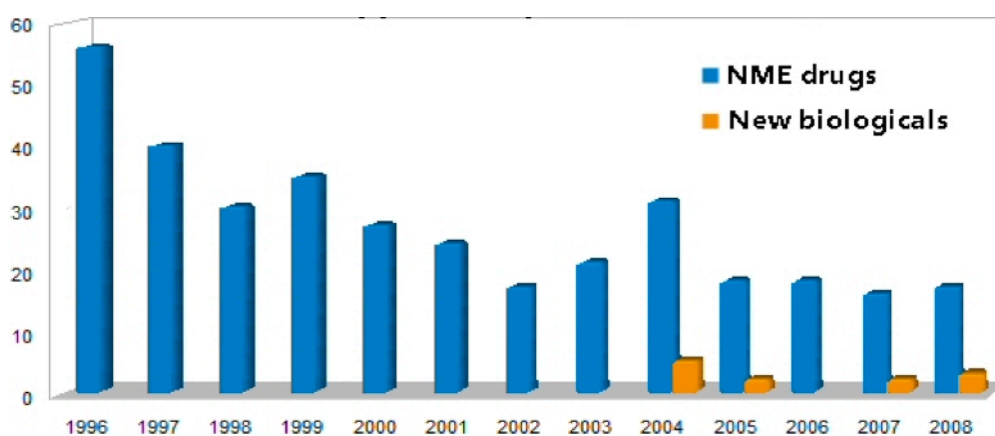


Figure n°3 | **NMEs and Biologicals approved by the FDA between 1996 and 2008.** Similar trends are observed for submissions and approvals at regulatory agencies worldwide. (FDA, 2009).

Figure 4 shows the trends of the global R & D expenditure, development times, sales and NME output between 1994 and 2003. Those trends are emphasized because of high attrition rates leading to unnecessary waste of money and time, preventing effective drug development.

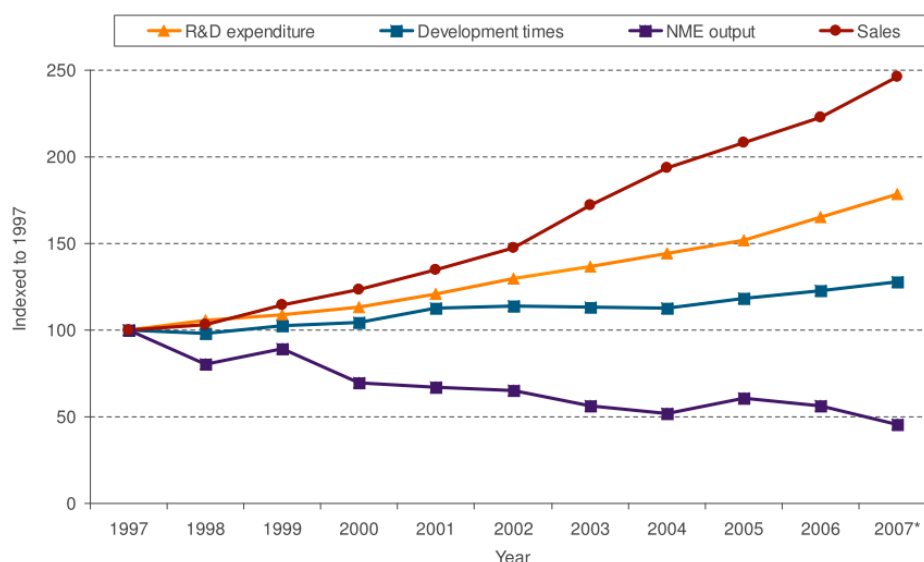


Figure n°4| Global R & D Expenditure, Development Times, Global pharmaceutical Sales, New Molecular Entity (NME) Output: 1994-2007. (CMR International & IMS Health, 2010).

As an example, according to a survey done by *Accenture* in 2003, a large pharmaceutical company like *Pfizer*, with revenues of approximately US \$45 billion in 2003, would need to generate about nine high-quality NCEs per year in order to remain a growing company on the basis of its current revenues and its desired percentage growth (*Accenture consulting*, 2001).

### 1.1.1.3 Attrition rate

The high cost for developing a new medicine is not only driven by the increasing clinical development times but also by the high rate of clinical failures. The vast majority of attrition occurs in late stages of clinical development (i.e. Phases IIb and III), where most of the costs are incurred. *Figure 5* estimates clinical phase transition and clinical approval probabilities for First-In-Man (FIM) compounds in the

development pipelines of the fifty largest pharmaceutical companies, between two time periods: 1993 – 1998 and 1999 – 2004.

The clinical approval success rates and phase transition probabilities vary significantly by type of drugs (twice as much for biologics compared to NCEs) and by therapeutic areas (oncology, cardiology, CNS, etc...) (Kola, I. & Landis, J., 2004). Despite efforts to improve the predictability and likelihood of overall clinical approval success, only one-in-six First-In-Man (FIM) compounds eventually obtained marketing approval during both the 1993-1998 and the 1999-2004 periods (Tufts, 2010). In other words, the average clinical approval success rate of drugs that enter clinical testing was approximately 16%, in the United States (DiMasi & al., 2010). However, one may notice that clinical transition probabilities slightly decline between Phase I and Phase II, and between Phase II and Phase III, suggesting that drug sponsors are becoming more aggressive about terminating unpromising candidates earlier, enabling them to re-direct increasingly scarce R&D resources to more promising drug development programs (Kaitin, K.I. & al., 2010). Although major progress has been made in the drug discovery process in the recent years (e.g. by focusing on high-throughput screening and combinatorial chemistry techniques), this has thus far not led to a proportional increase in the number of NMEs passing through the different phases of preclinical and clinical drug development (Kola, I. & Landis, J., 2004).

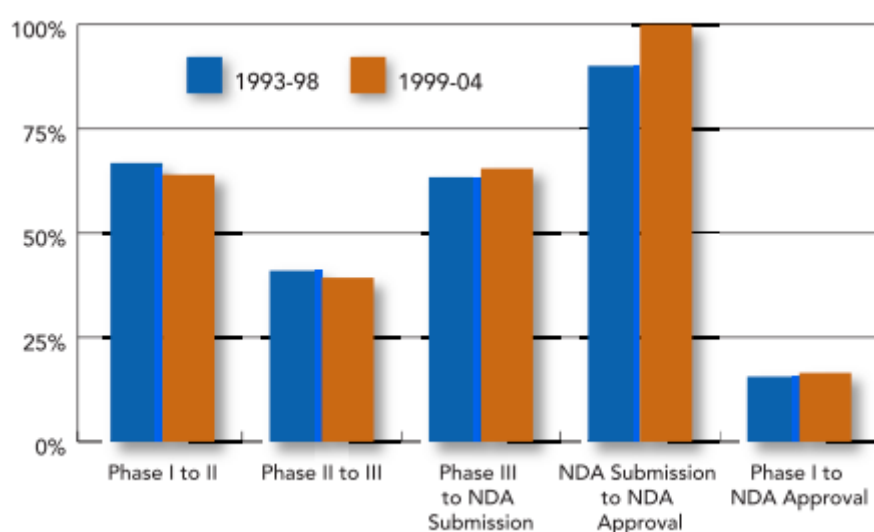


Figure n°5 | **Transition probabilities between phases of drug development of the 50 largest pharmaceutical companies.** (Tufts Center for the Study of Drug Development, 2010).

#### 1.1.1.4 Reasons for development failures

One of the reasons of the lower rate of success in the past few years may be explained, at least in part, by the fact that pharma companies are currently trying to treat complex diseases, that they are competing with high standard of care and also because Regulatory Authorities have become more demanding (Kola, I. & Landis, J., 2004). Whatever the reasons, drug sponsors have now to deal with these features. It is therefore instructive to examine factors that impact R&D success since it can help identify strategies to improve the efficiency of drug development (Kola, I. & Landis, J., 2004). *Figure 6* illustrates the reasons why compounds (all routes of administration confounded) undergo attrition and how this has changed over time. Attrition rates due to poor PK profiles and bioavailability (accounting for 40% of all attrition in 1991) have significantly reduced by 2000 (contributing to less than 10%). In 2000, the major causes of attrition in the clinic were lack of efficacy (accounting for approximately 30% of failure) and safety (toxicology and clinical safety accounting for a further approximately 30%) (Kola, I. & Landis, J., 2004). Although toxicology causes of failure decrease with subsequent development phase, efficiency causes of failure reach a peak in phase III and clinical safety increase with the number of people taking the drug (data not shown). The reason for up to 90% failure in clinical development is both related to the target (e.g. lack of efficacy, mechanism-related toxicology) and to the compound (e.g. pharmacokinetics, chemistry related toxicology).

The reduction of PK and bioavailability failures is explained by a better understanding of PK parameters in appropriate preclinical *in-vitro* and *in-vivo* models. However, one should note that those figures stand for drugs in general, of which most of them are orally administered. Indeed, *in-vitro* and *in-vivo* tests performed to obtain early ADME (Absorption – Distribution – Metabolisation - Elimination) parameters in oral compounds are not similar to those for topically administered compounds. For instance, there is no data showing evidence that PK and bioavailability hurdles have been overcome yet in dermatology. Besides, failures due to lack of efficacy and safety reflect the need to develop more predictive animal models where possible, and more importantly, the need to develop experimental medicine paradigm that are more predictive of outcomes and to carry out such proof-of-concept clinical trials much earlier in development (Kola, I. & Landis, J., 2004). Although there are no such figures

available for topical drugs developed in dermatology, those trends highlight actual issues in drug development, that is, the inability to predict these failures before human testing or in early clinical trials.

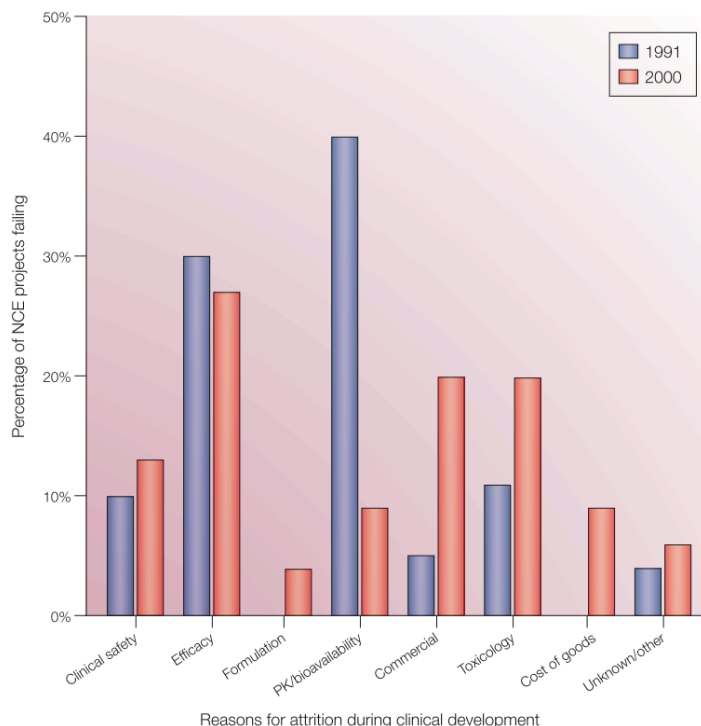


Figure n°6 | **Reasons for attrition between 1991 and 2000.** Reasons are expressed as a percentage of all drug projects stopped during clinical development. *NCE*: New Chemical Entity. *PK*: Pharmacokinetics. (Kola, I. & Landis, J., 2004).

## 1.1.2 Changing development paradigm

### 1.1.2.1 Learn-and-confirm paradigm proposed by Sheiner

Each phase of the development process is designed to increase the level of knowledge, thereby enabling assessment of the probability of success for an NME. An efficient and rational drug development program is based on the '*learn-and-confirm*' paradigm, as proposed by Sheiner, L.B. in 1997 (Figure 7). The main learning phases should be phase I and II, whereas phase III studies should merely have a confirmatory purpose.

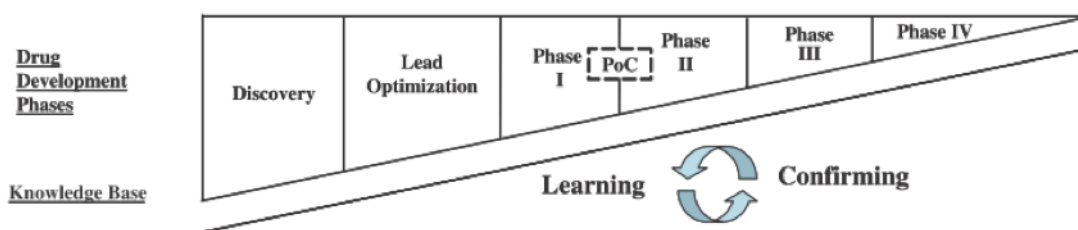


Figure n°7 | The learn-and-confirm paradigm in drug development. POC: proof of concept. (Sheiner, L.B., 1997).

### 1.1.2.2 The FDA's critical path challenge

In 2004, the FDA published a “White paper” entitled ‘*Challenge and Opportunities on the Critical Path to New Medical Products*’. Facing this ‘*productivity crisis*’, the FDA expressed their concerns by saying that “*excessive development costs are preventing new life-saving medicines reaching the patient at an affordable price*” (FDA, 2004). Indeed, such R&D expenditure may discourage investment in more innovative, risky approaches, as well as in therapeutics for diseases that represent smaller markets.

Orloff, the vice president of development for lead innovation projects at Novartis Pharma said, in 2006, that such trends “*are simply unsustainable over the long term (...) So while the drug development model used today has served us well in the past and indeed has delivered products that save and improve patients' lives, it's clear that we can't afford to be complacent, that we have to continuously innovate and evolve*”. This statement joined the FDA who said that “*new publicly available scientific and technical tools including assays, standards, computer modelling techniques, biomarkers, and clinical endpoints (...) are urgently needed to improve predictability and efficiency along the critical path (...) for development of safe products that benefit patients*” (FDA, 2004). Their diagnosis was that, although tremendous advances in basic scientific knowledge have been made, this knowledge has not been translated into practical tools that enable the development of new medicines. Besides, rising societal demands for greater certainty about the outcomes of drug therapy are occurring at a time while the pharmaceutical industry is experiencing difficulty in sustaining innovation.

This 2004 critical path report led to the FDA program called the '*Critical Path Initiative*' (CPI), which attempted to find ways to adapt the drug development process and incorporate advances in biomedical research and technology into a new development model (*Figure 8*).

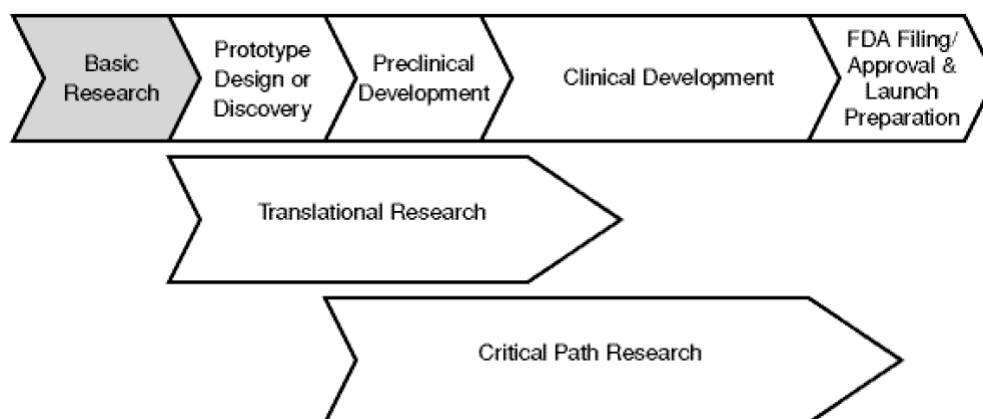


Figure n°8 | **Research support for product development.** ('Discovery' is applicable for molecules; 'Prototype Design' for device) (FDA, 2004).

The FDA identified the 'Critical Path' as the process that encompasses drug development process from the identification of a drug candidate for development to the marketing approval (FDA, 2004).

Indeed, in the traditional R&D model, basic research was separated from clinical development, molecules were "thrown over the fence" for safety testing and clinical trials. Conversely, Translational Medicine aims at integrating R&D by removing these fences and stimulating "bench to bedside" research. *Basic research* provides the foundation for product development. *Critical path research* is directed toward improving the product development process itself by establishing new evaluation tools. *Translational research*, which is also called '*experimental medicine*' or simply '*clinical pharmacology*' is concerned with moving basic discoveries from concept into early clinical evaluation (Wehling, M. 2006). Translational medicine groups would stand in-between research unit and development unit and facilitate the interaction between basic research and clinical medicine. Indeed, improvement in this part of the process is an essential step in modernizing drug development (Woodcock, J., Woosley, R., 2008).



The Critical Path of drug development may be subdivided into 3 dimensions. Once a candidate compound emerges from a drug discovery program, it must successfully complete a series of evaluations of its potential safety and efficacy and must be amenable to mass production (*Figure 9*) (Woodcock, J., Woosley, R., 2008).

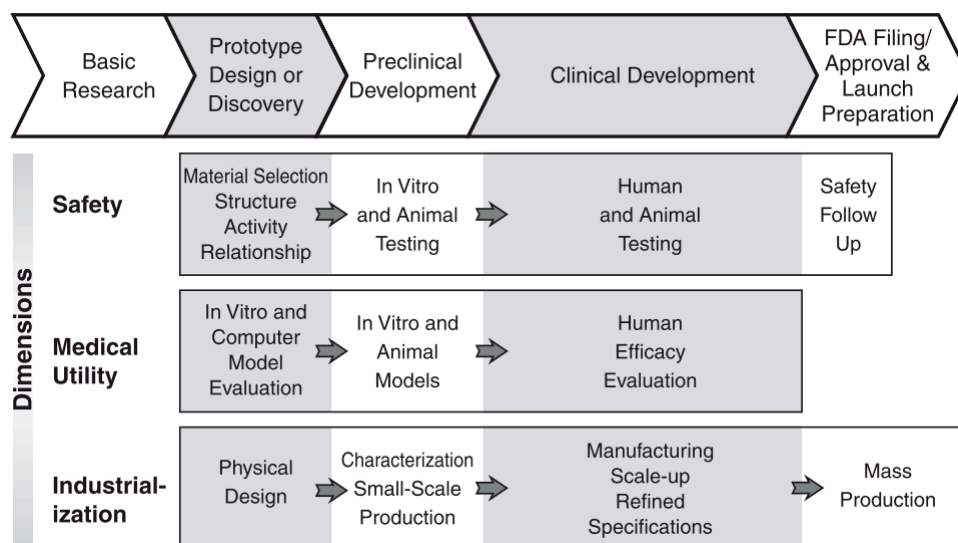


Figure n°9 | **Working in Three Dimensions along the Critical Path.** (FDA, 2004).

### 1.1.2.3 Critical path initiative

In March 2006, the FDA issued its '*Critical Path Opportunities Report & List*' (FDA, 2006a; FDA, 2006b), outlining the ways among which the industry can start to address such productivity and safety issues. Specifically, the FDA outlines distinct areas of research into six topics, determined to hold the greatest potential to impact drug development productivity:

- *Better evaluation tools – Developing new biomarkers and disease models*
- *Streamlining clinical trials*
- *Harnessing bioinformatics*
- *Moving manufacturing into the 21<sup>th</sup> century*
- *Developing products to address urgent public health needs*
- *Specific at-risk populations – Paediatrics*

In addition, the FDA pointed out the need for close collaboration among scientists, industry and the academic research community to take up the specific projects in each of these topics.

Only the two first categories will be tackled in this thesis. After introducing the rationale and hurdles found in the development of topical drugs in the treatment of psoriasis, we will focus on what has already been achieved in research and further objectives toward the improvement of anti-psoriatic topical NCE screening in early drug development phases.

Indeed the current challenges in psoriasis disease are:

- To obtain more predictable and reliable pre-clinical data (adequate models)
- To integrate new tools in pre-clinical / early clinical development in order to identify and validate PK/PD biomarkers
- To integrate such tools in early phase I experimental clinical trials

## 1.2 Rationale and challenges in drug discovery and development in psoriasis

### 1.2.1 Generalities on psoriasis

#### 1.2.1.1 Epidemiologic & clinical features

*“Lepra is easily distinguished from most other eruptions: from Psoriasis by the regular form of the patches, which in the latter disease are always irregular, and in which, also, the borders are neither elevated nor inflamed...”* (Bateman, T., 1836). Thanks to the first description of psoriasis made by a British dermatologist *Robert Willian*, psoriasis became a disease entity that is separated from leprosy, after hundreds of years of confusion.

Psoriasis is a common, chronic inflammatory skin disorder, affecting 0.6 to 4.8% of the general population (Naldi, L., 2004). The prevalence of psoriasis varies greatly between people of different ethnic backgrounds: this disease is more common in Caucasian people. Also the geographic latitude impacts the prevalence of the disease: it is more frequent in northern countries (accounting for 2-3% of the northern European population) than in equatorial countries, maybe because the sunlight has a beneficial effect on the disease (Griffiths, C.E., 2007). Initial outbreaks typically affect individuals in their twenties, but can occur at any age (Roberson, E.D.O. & Bowcock A.M., 2010). Psoriasis affects equally men and women. Moreover, there is a genetic contribution to psoriasis. For instance, one third of individuals with the commonest form of psoriasis have a first degree relative who is affected too.

The commonest clinical variant *Psoriasis Vulgaris*, also called chronic plaque psoriasis, accounts for 85 to 90% of all psoriasis cases (Griffiths, C.E., 2007). The other types of psoriasis, such as guttata, erythrodermic, exfoliative, inverse, pustular or palmo plantar, infected or ulcerated psoriasis will not be studied in this thesis.

The disease usually presents with plaques that occur on the extensor extremities (elbows and knees), lumbosacral region, umbilicus and scalp (*Figure 10*). Plaques are usually raised, well demarcated, erythematous and with adherent silvery scales (*Figure 11*) (Nestle, F.O. & al., 2009). The scales result from hyper-proliferative

epidermis with incomplete maturation of keratinocytes. For instance, keratinocytes of psoriatic skin reach the surface of the skin from the basal layer in as few as 6-8 days compared to approximately 30 days in normal skin. Regardless of the specific underlying pathogenesis, psoriasis is characterised by deregulated epidermal acanthosis<sup>1</sup> in combination with dermal and epidermal leukocytic infiltration and dilation of blood vessels (Danilenko, M.D., 2008). As many as 10-30% of psoriasis patients develop an inflammatory arthritis termed *psoriasis arthritis* which is progressive and leads to destruction of the joints if not treated aggressively (Roberson, E.D.O. & Bowcock, A.M., 2010).

Most patients (65-80%) suffer from mild psoriasis (< 3% of Body Surface Area<sup>2</sup> (BSA)) (Table I). The modified appearance of the skin often embarrasses patients and does sometimes affect their social interactions and employments. Moreover, treatments are not devoid of side effects. Although psoriasis is not a life-threatening disease, all these factors may have a substantial negative impact on patient quality of life.



Figure n°10| **Chronic plaque psoriasis.**  
(Griffiths, C.E., 2007)



Figure n°11| **Single plaque of psoriasis, well demarcated and heavily scaled.** (Griffiths, C.E., 2007)

Severity	Body surface area affected (%)	Prevalence in patients with psoriasis (%)
Mild	<3	65–80
Moderate	3–10	20–25
Severe	>10	5–10

Table I| **Psoriasis: disease severity and prevalence.**  
(National Psoriasis Foundation)

<sup>1</sup> **Acanthosis** is diffuse epidermal hyperplasia. It implies increased thickness of the epidermis.

<sup>2</sup> **Body Surface Area** (BSA) represents the surface of a human body. It defines the psoriasis disease severity.

<sup>3</sup> **Body Surface Area (BSA) Syndrome** is the surface of Cushing's body, one of the endocrine glands, caused by

The primary nature of the disease is still unclear. Questions remain unresolved, whether the *“primary cause is from an epithelial or immunologic disorder, the autoimmune cause of the inflammatory process, the relevance of cutaneous versus systemic factors, and the role of genetic versus environmental influences on disease initiation, progression, and response to therapy”* (Nestle, F.O., 2009).

### **1.2.1.2 Aetiology and Pathophysiology**

It is essential to take into account predisposing factors and to understand disease initiation and disease maintenance for an appropriate strategy in treatment approaches.

Psoriasis disease is initiated with interplay between genetic susceptibility and environmental factors. Psoriasis appears to arise through multiple genetic risk factors, which means that not just one polymorphism is responsible for the disease (Griffiths, C.E. & al., 2007). *PSORS1* would be the major genetic determinant of psoriasis accounting for 35-50% of the heritability of the disease (Trembath, R.C. & al., 1997). Environmental factors include infection by a micro-organism ( $\beta$ -haemolytic streptococcus, HIV), drugs (including  $\beta$ -blockers and lithium), stress, trauma and smoking (Nestle, F.O. & al., 2009).

Psoriasis disease presents immuno-inflammatory and micro-vascular features. Scientific evidence accumulated in the past 40 years, has established the presence of increased number of immune cells in psoriatic lesions, providing support for a functional role of a dysregulated immune system in psoriasis disease. It was shown that the innate as well as the adaptive immunity is crucial in the initiation and maintenance of psoriatic plaques (Nestle, F.O. & al., 2009). Thus, psoriatic lesions probably evolve as an interface between cells (e.g. keratinocytes, macrophages, Langerhans, dendritic and T cells), mediators (e.g. cytokines including interferon, TNF, IL-12, IL-17, IL-20, IL-23, pro-inflammatory cytokines such as TNF $\alpha$ , IL-1, IL-6 and chemokines including CXCL8, CXCL10, CXCL20) and antimicrobial peptides such as LL-37,  $\beta$ -defensins, and psoriasin (S100A7) of the immune system (*Figure 12*). Moreover, the level of the Vascular Endothelial Growth Factor (VEGF) is significantly raised in

plaques of psoriasis. Indeed, psoriatic microvasculature is characterised by tortuous and leaky blood vessels that facilitate leukocytes migration into inflamed skin. VEGF could be responsible for these vascular changes in psoriasis (Nestle, F.O. & al., 2009).

Therefore, some components from the inflammatory infiltrate or the vascular growth detailed above represent potential future therapeutic targets or biomarkers.

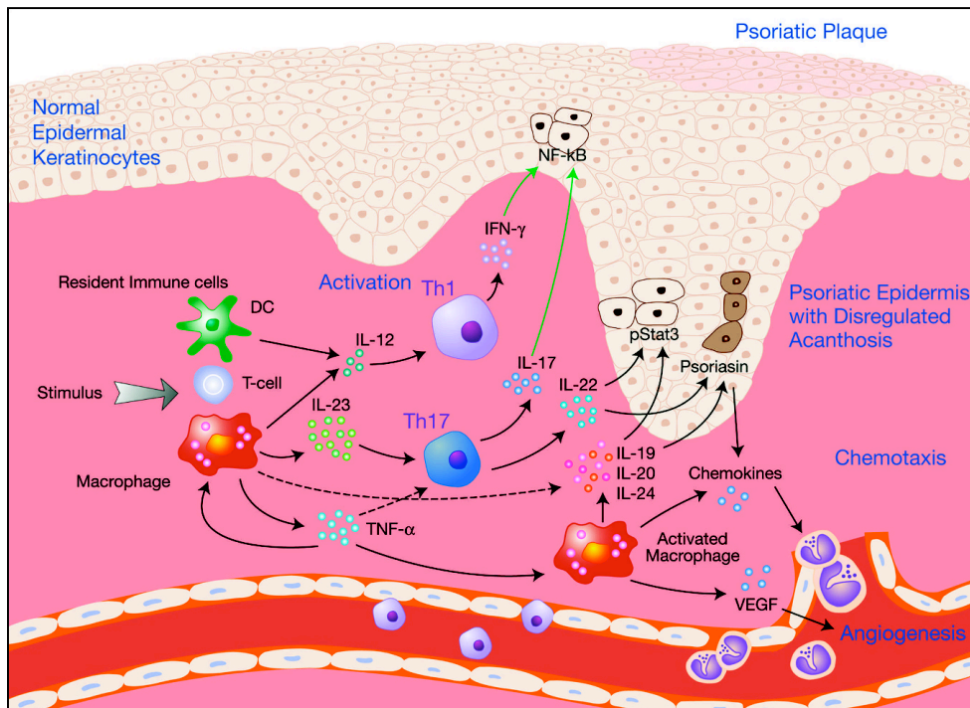


Figure n°12| **Immunoinflammatory and micro-vascular features of psoriasis.** The interface between epidermal keratinocytes, the innate and adaptive immune system, the vascular endothelium and their effectors shapes the psoriatic inflammatory process. (Danilenko, M.D., 2008).

Although current data do not support the notion that psoriasis is an autoimmune disease, psoriasis is probably *“best placed within a spectrum of autoimmune-related diseases characterised by chronic inflammation in the absence of known infectious agents or antigens”* (Davidson, A. & Diamond, B, 2001). In addition to being a skin inflammatory disease, psoriasis is also a systemic inflammatory disease. For instance, psoriasis shares important systemic manifestations with other chronic diseases, such as Crohn’s disease and diabetes mellitus. These shared conditions are the metabolic syndrome, depression, and cancer (Griffiths, C.E., 2007).

A better understanding of the pathophysiology of psoriasis disease would allow the setting of (1) disease prevention measures targeting genetic susceptibility,

environmental factors as well as disease mechanisms; (2) a more pathogenesis-based approach rather than previous empirical approaches in disease treatment; (3) the identification of biomarkers in order to improve the ability to predict disease responses and natural history; and finally (4) a better assessment of the mechanism leading to co-morbidities and potentially the finding of concomitant therapies preventing those co-morbidities (*Figure 13*).

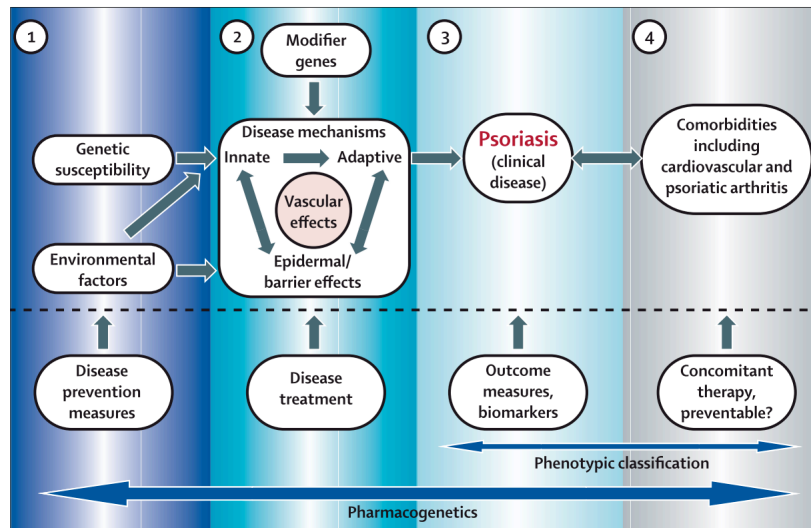


Figure n°1.13| **The march of psoriasis.** This schematic combines information that is currently accepted on psoriasis (upper dotted-line) and clinical issues and intervention strategies that are under active investigation (boxes under dotted-line). The use of validated pharmacogenetic and phenotypic classification tools would help resolving issues and finding strategies. (*Griffiths, C.E., 2007*).

## 1.2.2 Management of psoriasis disease: A focus on topical therapies

### 1.2.2.1 Present state of marketed topical therapies in psoriasis

Knowing that psoriasis is (so far) an incurable skin disorder, the goal of an anti-psoriatic therapy is to clear skin lesions and prevent their recurrence. A wide range of therapeutic options is available for patients, including topical agents, phototherapy, conventional oral systemic agents and biologics. The choice between these agents depends on disease severity (*Figure 14*). Whereas biologics revolutionised care management of patient with severe psoriasis, topical monotherapy remains the mainstay of psoriasis treatment for most patients, especially for mild to moderate cases. Phototherapy is generally used in association, for moderate disease; systemic agents for moderate to severe disease and biologics are generally reserved for severe cases. However, the treatment choice is not straightforward. Physicians are now

trying to individualise treatment management on the basis of the “*nature and extent of disease, anatomical locations, quality-of-life implications, coexistent psoriatic arthritis, triggering factors (such as infections, medications, and stress), and the patient’s commitment to therapy*” (Menter, A. & Griffiths, C.E.M., 2007). For instance, a patient presenting moderate psoriasis (i.e. 3 to 10% of BSA affected) whose psychosocial life is greatly affected (i.e. embarrassment because of appearances, employment difficulties...) will be treated with a stronger therapy (e.g. biologicals, potentially in combination with a topical agent, instead of topical therapy alone).

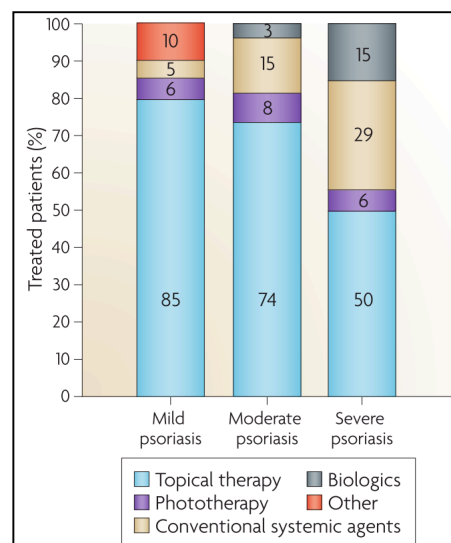


Figure n°14 | Type of therapeutics prescribed to patients with psoriasis depending on disease severity. (Melnikova, I., 2009)

In the past twenty years, the development of topical therapies has greatly improved the ability to safely and effectively treat psoriasis. The preferred topical agents are corticosteroids, vitamins D and vitamins A derivatives.

Very potent **topical glucocorticoids (GCs)** such as clobetasol propionate 0.05%, or potent GCs such as bethametasone dipropionate 0.5%, remain the most commonly prescribed agents for psoriasis. However, their long-term side effects including iatrogenic Cushing syndrome<sup>3</sup>, skin atrophy, telangiectasis<sup>4</sup> and tachyphylaxis<sup>5</sup>, limit their use (Lebwohl; M. & Ali, S., 2001).

<sup>3</sup> **Iatrogenic Cushing syndrome** is a form of Cushing’s syndrome (also called hypercorticism) caused by administration of glucocorticoid (also called corticosteroid).

<sup>4</sup> **Telangiectasis:** Chronic dilation of groups of capillaries causing elevated dark red blotches on the skin.



The use of **topical vitamin D analogs** for the psoriasis indication has been discovered by chance. While a patient was being treated with calcitriol for osteoporosis, his psoriasis got cured (Morimoto, S. & al., 1985). The first vitamin D derivative approved for psoriasis was calcipotriol in the early 1990s (Lebwohl; M. & Ali, S., 2001). Vitamin D analogues are now first-line in long-term treatment: they are effective and relatively well tolerated beside skin irritation and potential risks of hypercalcemia. Calcipotriol 50µg/g, calcitriol 3µg/g and tacalcitol 4µg/g are commonly used vitamin D analogs. Calcipotriol combined with betamethasone dipropionate 0.05% has demonstrated superiority over either agent alone.

**Topical Retinoids**, such as tazaroten, are vitamin A derivatives. They are quite efficient but unfortunately, when used in monotherapy, a significant proportion of patients develop irritation at the site of application (Lebwohl, M. & Ali, S., 2001). Moreover, all retinoids are teratogens.

Topical immunomodulators such as calcineurin inhibitors (*e.g.* tacrolimus 0.03%, 0.1% ointment and pimecrolimus 1% cream) are not often used because of their observed lack of efficacy. This might be explained by their relatively high molecular weight (800Da) preventing sufficient penetration through the corneal layer (500Da rule; *cf* 3.1.1.) (Bos, J.D. & Meinardi, M.M.H.M., 2000).

The use of **other topical therapies** such as salicylic acid, anthralin and tar-based preparation has declined in favour of the more efficient corticosteroids and non-corticosteroids therapies concisely described above (Lebwohl, M. & Ali, S., 2001).

Topical treatments can be combined with each other (*e.g.* vitamin D analogs + GCs); vitamin D analogs or GCs with photo(chemo)therapy (UVB or Psoralen + UVA), conventional systemic therapies or with biologicals. *Table II* describes major characteristics of topical therapies in psoriasis in use today.

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<sup>5</sup> **Tachyphylaxis:** describe a rapid decrease in the response to a drug after repeated doses over a short period of time. Increasing the dose of the drug will not increase the pharmacological response. Example: long-term corticosteroids.

Topical entity	Use	Remittive suppressive	Problems	Combinations
Anthralin	Short contact Day care	Remittive	Staining skin/clothes Skin irritation Availability	UVB/PUVA
Tar	Once daily Day care	Suppressive	Carcinogenicity Odor Availability	UVB
Glucocorticoids	Once daily Class I-II in folds Class III-IV on extensor surfaces	Suppressive	Skin atrophy Teleangiectasia Tachyphylaxis	Vitamin D derivatives
Vitamin D derivatives	Twice daily	Suppressive	Skin irritation Hypercalcemia	Corticosteroids
Vitamin A derivatives	Twice daily	Suppressive	Teratogenicity Retinoid erythema	Systemic therapy
Calcineurin inhibitors	Twice daily	Suppressive	Lack of efficacy Burning	

Table II | Major characteristics of topical treatments in psoriasis in use today (Bos, J.D. & Spuls, P.I., 2008).

### 1.2.2.2 Consensus, guidelines, and evidence-based dermatology:

A Cochrane review was published in 2009 on “*Topical treatments for chronic plaque psoriasis*”. The evidence was based on 131 studies that included 21,448 people and compared all the above-mentioned psoriatic treatments. The main conclusion was that corticosteroids perform as well as vitamin D analogues and are associated with a lower incidence of local adverse events but that further research was required to inform long-term maintenance treatment. Moreover, combined treatment with corticosteroids/vitamin D derivatives performed significantly better than both used alone (Mason, A.R. & al. 2009).

### 1.2.2.3 New directions for future topical therapy of psoriasis

Drug discovery approaches for topical agents until today were rather more empirical than physiopathologically-based. For instance, the precise mechanisms by which topical agents interfere in psoriasis are often not known (Bos, J.D. & Spuls, P.I., 2008). Besides, we may ask ourselves if it is worth developing new topical therapies since the ones we currently use in clinic are relatively efficient and safe, although significantly less efficient than systemic agents and biologics.

Although topical therapies stand for only one third of the biologics sales (*Figure 15a*), topical agents still dominate the R & D landscape for psoriasis, representing 42%

of programs in the development (*Figure 15b*). Also, topical therapeutics (as well as oral agents) offer cost saving over biologics.

It is very challenging to discover and develop better topical agents than the existing ones. The current approach in the development of new topical therapies in psoriasis is mainly characterised by elaborating new formulations and combination of existing corticosteroids/ vitamin D derivatives in order to enhance efficacy as well as patient compliance. Besides, innovative corticosteroids and vitamin D receptor ligand may have a potential for psoriasis by improving their safety and/ or efficacy. Also, as mentioned above, treatment in psoriasis would benefit from pathophysiology-based agents that, for instance, operate on the natural immune system pathways in psoriasis (*Table III*). An extensive list of topical treatments in the pipeline can be found on *The National Psoriasis Foundation website*.

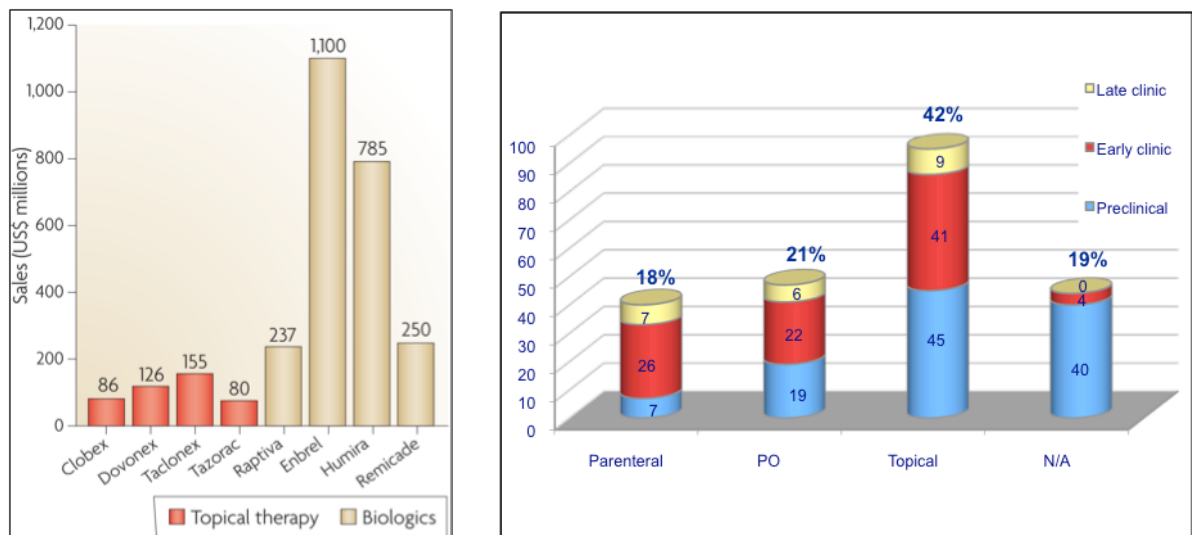


Figure n°15| a. **2008 sales of selected psoriasis drugs.** (Principes: molecule) Clobex®: Clobetazol propionate; Dovonex® (= Daivonex®): calcipotriol; Taclonex®: calcipotriol + betamethasone dipropionate; Tazorac®: Tazaroten; Raptiva®: efalizumab; Enbrel®: etanercept; Humira®: Adalimumab; Remicade®: Infliximab. (*IMS Health and Evaluate Pharma*). b. **Psoriasis drug pipeline by route of administration.** 'Preclinical': before the clinic; 'Early clinic': Phase I, IIa; 'Late clinic': Phase IIb/III. (*Galderma, 2010*).

New formulation of corticosteroids and vitamin D derivatives  
 Combination corticosteroids + vitamin D derivatives  
 Innovative corticosteroids and their receptor ligands  
 New vitamin D receptor ligands  
 Agents aimed at Toll-like receptors  
 Agents inhibiting pro-inflammatory cytokines (IL-12, IL 23, IL-17, TNFα)

Table III | **Current approaches in topical therapeutics of psoriasis.**  
 (Adapted from Bos, J.D. & Spuls, P.I., 2008; Galderma, 2010).

One of the long-standing goals of drug research is to develop corticosteroids with an improved benefit/ risk ratio by dissociating the wanted (i.e. immunomodulation by silencing of pro-inflammatory genes such as for TNF $\alpha$ ) from the unwanted (adverse reactions) of corticosteroids. The recently identified *selective GC receptor agonists*, which are non-steroidal GC receptor agonists, show great potential in reducing side effects of GC although being equipotent regarding immunosuppression. For example, *ZK-245186* is a molecule developed for atopic dermatitis but which also has a potential for psoriasis (Schoepe, S. & al., 2006).

Vitamin D analogs exert their effects by binding to the vitamin D receptor (VDR) that belongs to the steroid/ thyroid hormone nuclear receptor super-family (Bos, J.D. & Spuls, P.I., 2008). They have an effect on cell differentiation, proliferation, immunomodulation and mineral homeostasis (Bos, J.D. & Spuls, P.I., 2008). The objective is to make vitamin D receptor ligands more selective in order to reduce the risk of irritation and hypercalcaemia (Ma, Y. & al., 2006).

Toll-like receptors (TLRs) are crucial in early host defence against invading pathogens. Since an innate immune response is initiated by interaction between host cells and environmental triggers, a new approach to the topical therapy of psoriasis would be to find *TLR inhibitors* (Bos, J.D. & Spuls, P.I., 2008). There is no such molecule in development for topical application yet.

Another *pathophysiogenesis-based approach* would be to interfere with the intracellular pathways involved in pro-inflammatory cytokines production.

For instance, several kinase inhibitors are in various stage of development. The most promising ones are *Janus-Activated-Kinase (JAK) inhibitors* (e.g. ruxolitinib, phase IIb and tasocitinib, phase IIa). A *P38 mitogen-activated protein kinase (MAPK)* inhibitor is in preclinical development. However, the development of other kinase inhibitors has already been stopped, in most cases due to liver toxicity.

Other inhibitors such as the Phosphodiesterase (PDE4) inhibitors (e.g. AN2728, phase IIb) and PDE7 inhibitors (e.g. ASB-16165, phase I) influence pro-inflammatory cytokines production, including TNF $\alpha$ , IL-12 and IL-23 (Melnikova, I., 2009).

### 1.3 Limitations and perspectives in developing new topical therapies for psoriasis

A number of critical points must be considered in drug discovery and early development of topical products, related to (1) the route of administration, (2) the relevance and predictability of current non-clinical models to human, (3) the suitability of dermal evaluation methods in predicting human PK and/ or PD parameters in the skin, as well as (4) the local tolerance assessment.

*Note:* Systemic toxicity will not be part of this thesis since it is well defined in guidelines, in opposition to local tolerance.

#### 1.3.1 Particularities and concerns regarding the route of administration

Like any other route of administration, the topical route has its own specificities and concerns, independently of the treated disease. Human normal skin is defined by four distinctive layers, that is, stratum corneum, epidermis, dermis and hypo-dermis (Figure 16):

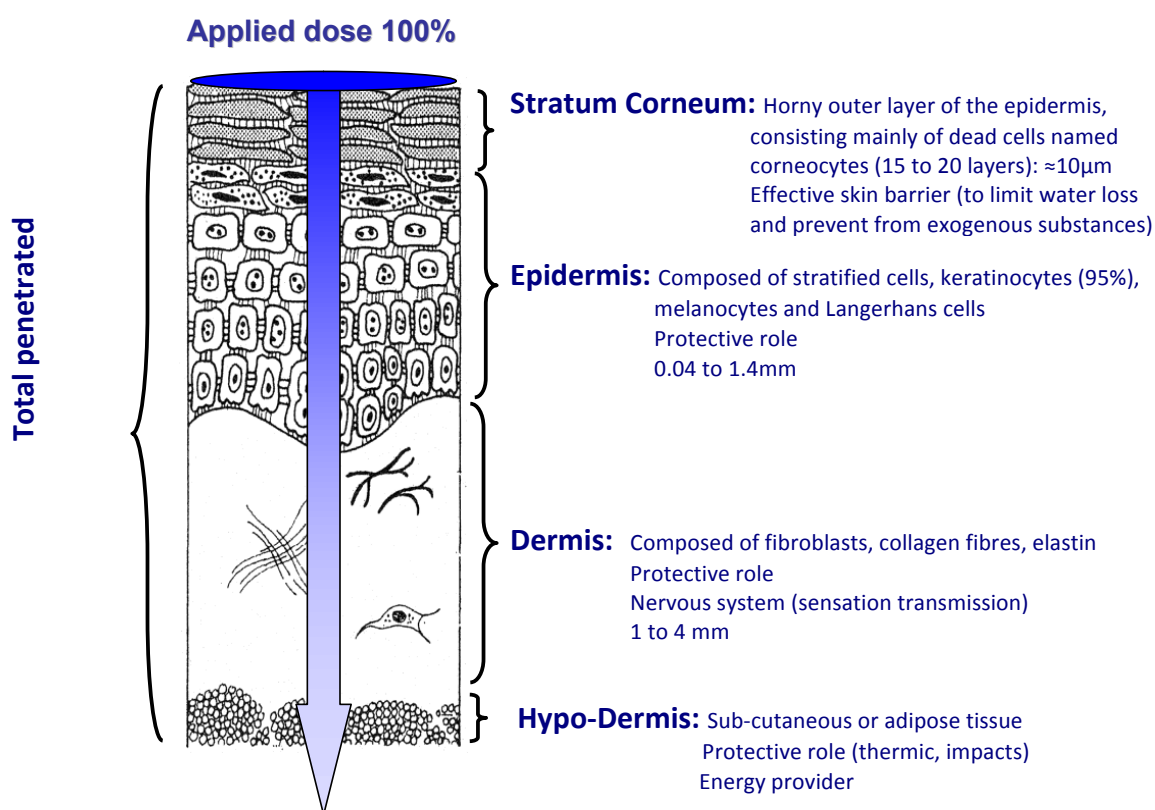


Figure n°16 | Structure of human healthy skin and the roles of each separate layer. (Adapted from Galderma's documentation).

Skin is considered as an organ and represents approximately 15% of our body mass. Its main physiological functions are: protection (to UV, chemical and mechanical factors, pathogens), hydration, thermoregulation, metabolic activity and secretion (sweat, sebum...). Understanding the physical structure of the skin is essential to considerate both its barrier function, the disruption mechanism caused by topical applied agents and also, for the comprehension of several skin disorders. For instance, skin disorders such as psoriasis affect the skin barrier to a certain extent (*cf* psoriasis clinical features, *section 1.2.1.1*). There are six factors/criteria that need to be accounted for when considering route of administration. These are described below:

#### **1.3.1.1 Type of molecule - Skin barrier & Topical delivery**

A topical drug penetrates into the skin by a passive mechanism according to Fick's laws, depending on its physicochemical properties (i.e. molecular weight, molecular size, the hydrophilic-lipophilic balance, the distribution of polar and non-polar parts in the molecule and the extent of the ionized state) (Forster, M. & al., 2009).

For instance, one of the major prerequisites for development of a compound in dermatology is that the molecular weight of the drug substance is less than 500Dalton. Indeed, the “500 Dalton rule” for skin penetration, established by J.D. Bos & M.M.H.M. Meinardi in 2000, applies for all molecules intended to cross the corneal layer. For example, as described previously, the lack of efficacy of calcineurin inhibitors previously mentioned may be explained by their relatively high molecular weight (i.e. 800Da), which makes transcorneal or transepidermal delivery next to impossible (Bos, J.D. & Spuls, P.I., 2008). In theory, such molecules could be administered systemically by subcutaneous, intramuscular or intravenous injections. Proteins, which have by definition a molecular weight expressed in several kDalton, cannot be delivered topically by a passive mechanism. However, this molecular weight limit can be bypassed by the use of active mechanisms. For instance, methods such as electroporation<sup>6</sup>, sonophoresis<sup>7</sup>, local thermal treatment, mechanical perforation of

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<sup>6</sup> **Electroporation** : Active mechanism that consists in applying « high voltage impulses during short time intervals to create temporary pores on the skin » (Forster, M. & al., 2009).

the stratum corneum or even micro-needles array have been developed to optimise transdermal delivery of large molecules (Forster, M. & al., 2009).

The stratum corneum (SC) is a heterogenous two-compartment system of flattened dead cells filled with keratins (named corneocytes) cells embedded in a multilamellar lipid matrix (mainly composed of neutral lipids and ceramides) (Herkenne, C. & al., 2008). The SC is lipophilic and contains 13% of water. It is considered to be the rate-controlling membrane for transport of xenobiotics across the skin. The following figure shows the main permeation pathways identified in the SC (*Figure 17*). The first pathway is the intercellular route and the second one is the intracellular (transcellular) route that crosses successive corneocytes and the lipid matrix. The third pathway (of little significance) is the route along the skin appendages (hair follicles and the sweats glands). Whereas pathway 2 presents significant resistance to permeation of a drug, pathway 1 is the most common route for drug permeation by its intercellular passage between the corneocytes.

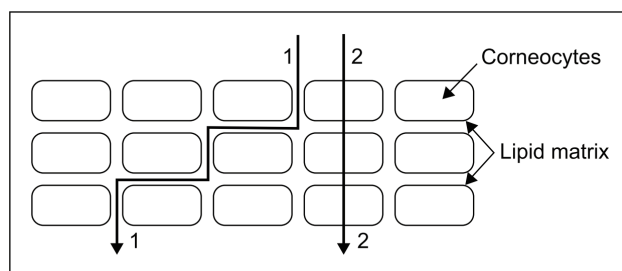


Figure n°17| **Main penetration routes across the non-viable stratum corneum.** Pathway 1: Intercellular way. Pathway 2: Transcellular way. (Forster, M. & al., 2009).

Thus, in addition to a low molecular weight and size, a new topical compound intended to cross the SC barrier should preferably be lipophilic as well. Therefore depending on the lipophilic or hydrophilic properties of a drug, it will “accumulate in the stratum corneum (lipophilic drugs), or stay on the surface (very hydrophilic drugs) or cross the skin (amphiphilic<sup>8</sup> drugs)” (Forster, M. & al., 2009).

<sup>7</sup> **Sonophoresis** : Active mechanism that uses « low frequency ultrasonic energy to disrupt lipid packaging in the SC creating aqueous pores, which improve drug delivery » (Forster, M. & al., 2009).

<sup>8</sup> **Amphiphilic drug**: Molecule having a polar, water-soluble group attached to a non-polar, water-insoluble hydrocarbon chain.

### 1.3.1.2 Percutaneous absorption & Formulation aspects

A broad range of formulations can be applied to the skin. The elaboration of a new formulation must take into account physicochemical properties of the drug substances but also the disease and the *in use* aspects. The most common formulations used in psoriasis are creams, ointment and lotions. More recently, other formulations have been developed in order to facilitate usage and potentially patient compliance (e.g. foams, sprays, and gels).

As introduced earlier, the formulation composition critically influences pharmacokinetic parameters of a drug. Indeed, the formulation of a topical agent is decisive regarding to the mechanisms by which a drug leaves its formulation and penetrates the skin up-to its site of action. For instance, the skin penetration of a drug can be enhanced by its formulation. The formulation itself can influence the solubility properties of a drug and its partition coefficient<sup>9</sup> (e.g. prodrug<sup>10</sup>, eutectic system<sup>11</sup>, complexation in cyclodextrins, chemical potential), but also the *stratum corneum* (e.g. the use of microemulsions) and the solubility of the drug as in encapsulation (e.g. nanoparticles, liposomes and analogue vehicles) (Forster, M. & al., 2009).

One issue encountered in dermatology is that when a new compound is not efficient, it is hard to determine whether the molecule really does not have any effect or whether the drug substance does not reach its site of action because of the formulation. The latter underlines the importance of the prediction of pre-clinical models before human testing in the choice of the right candidate. The following example is taken from Galderma's experience:

Clobetasol propionate (a high potent GC) is known to be very efficient on psoriasis. However, when its formulation was modified into a gel (while previously formulated in a lotion formulation), the drug did not show any clinical efficacy or side effects anymore. This was explained by the fact that the drug substance did not

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<sup>9</sup>**Partition:** "term applied to the distribution of a substance between two adjacent but different phases at equilibrium". For example, the partition between the skin and the formulation is described by the (stratum corneum/formulation) partition coefficient:  $K_m$  (Forster, M. & al., 2009).

<sup>10</sup>**Prodrug:** "Drug derivative with better solubility and transport properties in the SC than the parent drug; its use may be helpful for drugs with unfavourable partition coefficients" (Forster, M. & al., 2009).

<sup>11</sup>**Eutectic systems :** it has been stated that a "lower melting point for a drug influences positively the solubility and the pénétration into the SC". A good example is the EMLA® cream (Forster, M. & al., 2009).



penetrate, as scales compacted in the gel formulation, leading to the formation of a 'cake' (Figure 18). Indeed, the degree of penetration depends not only on the drug itself but also on the vehicle in which it is formulated and on the interaction between the vehicle and the skin (Forster, M. & al., 2009). If the formulation is inappropriate, any biological effect may be masked.

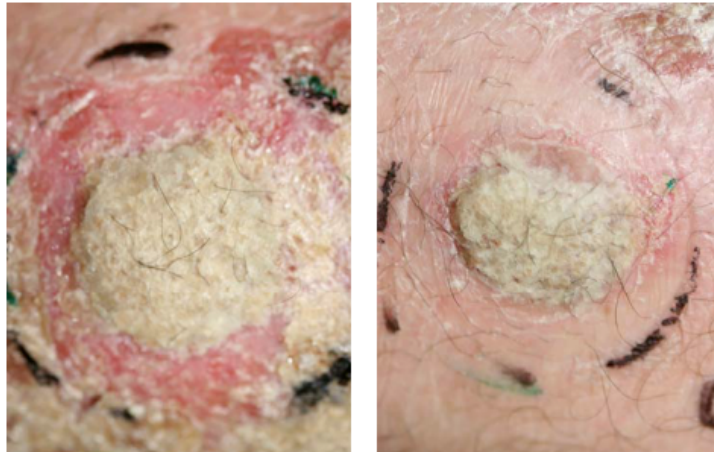


Figure n° 18| **Example of what could happen with an inappropriate formulation.** A 'cake' was formed because of the compaction of scales in the gel formulation. (*Galderma's documentation*)

### 1.3.1.3 Topical application on the skin

A specificity of dermatology is that the drug is directly applied on the pathological skin, which is itself the target. This remark underlines that topical agents have usually low skin metabolism and that they are devoid of hepatic first-pass metabolism.

Although effective for individual plaques, product application is time consuming, and compliance is a substantial issue (~39% of patients are noncompliant with their prescribed topical psoriasis therapy). Low compliance leads to lack of efficacy and therefore unsatisfaction, that is to say ~70% of patients, according to 3 large surveys (Finlay, A.Y. & al., 2004). In addition, the dosing may worsen this situation, i.e. some products require a twice-daily application, which can be seen as burdensome by patients. Moreover, the formulation's texture is usually not very pleasant and the quantity to be applied is consequent if the posology is respected. Finally topical therapies are not without side effects (e.g. skin atrophy, irritation..., cf section 1.2.2.1). Thus, it is important to individualise and simplify topical treatments

by using, for example, the appropriate bases: creams, lotions, foams, sprays, ointments, or gels in order to favour compliance.

#### **1.3.1.4 Sampling methods**

Although plasma PK is a good representative of the PK profile of a systemic drug, having systemic availability information for an applied topical agent may not properly reflect local cutaneous bioavailability (Herkenne, C., 2008). However, in dermatology, the easy access to the skin enables sampling with relatively low-invasive methods. Although more invasive than blood sampling, skin biopsy, tape stripping and microdialysis seem to be relatively well accepted by subjects in clinical trials (those methods will be widely explained in *section 1.4.2.3*). Such samplings provide PK information (such as proof of exposition for the tape-stripping and skin biopsy; and/or concentration time-profile for the microdialysis) and PD information on molecular, cellular and genomics features of psoriasis. Such information should facilitate the identification of disease-related and target-related biomarkers and the discovery of novel therapeutic targets (Petersen, T.K. & Sørensen, P., 2008).

#### **1.3.1.5 Regulatory guidelines**

From a regulatory point of view, researchers have to juggle guidelines that are usually written for oral route administration. Thus, they must be interpreted and translated for dermatological use. As an example, first PK *in-vitro* studies performed for a new topical compound are: human keratinocyte culture, reconstructed human epidermis, normal human skin (*Franz cells*) for liberation-penetration studies. For *in-vivo* studies, the choice of animals is different for topical products, compared to oral compounds. For example: mini-pigs are known to have the closest skin type to human skin. Despite scarce dermatological focused guidelines, a “*Guideline on clinical investigation of medicinal products indicated for the treatment of psoriasis*” has been issued by the *EMA* in 2004.

#### **1.3.1.6 Topical drug development time**

Finally, drug development time of topical products is, at least, as long as for oral administration drugs. Indeed, in addition to the assessment of systemic safety,

local (cutaneous) safety must be assessed as well. Moreover, safety requirements are very strict in dermatology. For instance, as topical dermatological therapies are usually indicated for mild to moderate skin diseases, any severe adverse event would be unacceptable in opposition to more severe diseases. The benefit/risk ratio has to be particularly favourable for topical products. Lastly, it is hard to discriminate drug efficacy from a vehicle effect. A vehicle effect differs from a placebo effect by its ability to moisturize skin lesions and potentially enhance skin recovery. A vehicle effect thus encompasses both placebo and formulation effects.

### 1.3.2 Limitations of current non-clinical models in psoriasis research

Although relevant and predictable non-clinical models in psoriasis would come in handy for psoriasis pathogenesis research and drug development, an experimental model that completely mimics the complex human disease is not yet available (Boehncke, W.H. & Schön, M.P., 2007). After presenting the two main psoriasis-related issues in experimental models, the relevance of such experimental models and predictability to humans will be assessed. The non-clinical models that will be tackled in the following section encompass *in-vitro*, *ex-vivo* and *in-vivo non clinical* models determining PK, PD, PK/PD and local tolerance before processing into the clinic (*table IV*).

First, since psoriasis is unique to human, there is **no naturally occurring disorder in non-human species** that reflects the complex phenotype of psoriasis and which responds to anti-psoriatic treatment (Schön, M.P., 2008). The searched pathogenic alterations in a non-clinical model would be “*chronic inflammatory erythrosquamous skin lesions underlined by epidermal hyperproliferation, altered differentiation, angiogenesis and a psoriasis-like infiltrate*” (Schön, M.P., 2008).

Second, psoriasis is a complex skin disorder, with **no single feature that is truly specific** to it. For example, “*metabolic and biochemical abnormalities*” are found in psoriasis but also in other inflammatory skin diseases, “*dysregulation of any given inflammatory mediator or adhesion molecule*” may also be found in diseases unrelated to psoriasis, “*genetic markers show only statistical associations with psoriasis*”, and “*established therapies are not exclusively effective in psoriasis*” (Schön, M.P., 2008).

	Existing non-Clinical models (low or medium throughput)		
	PD : Pharmacological activity models	Penetration / PK Skin delivery model	Local tolerance Models
<i>Ex-vivo</i>	- <i>Ex-vivo</i> viable normal human skin (organoculture)	- Human skin in diffusion cell (liberation/ penetration studies)	Not yet
<i>In-vitro</i>	- RHE* using normal human keratinocytes <sup>1</sup> - RHE using psoriatic keratinocytes overexpressing pro-inflammatory genes (TNF $\alpha$ , IFN $\gamma$ , and IL-8 <sup>2</sup> )	- Reconstructed human skin using normal human keratinocytes (skin delivery model)	Not yet
<i>In-vivo</i>	- TPA (induction of inflammation in rodents) - Mouse tail test (para-keratosis) - Full mice (spontaneous mutation, genetically engineered, immune transfer) - Mice humanisation (xenotransplantation)	- Mini-Pig	- Mouse ear oedema - Mini pig (mini zones) - Rabbit

Table IV| **Existing non-clinical models in psoriasis.** \*RHE: Reconstructed Human Epidermis. (Adapted from Galderma documentation. <sup>1</sup> Danilenko, M.D., 2008; <sup>2</sup> Barker, C.L. & al., 2004).

All these models have their advantages and limits in gathering relevant information for the selection of the best drug candidate that will progress into clinical evaluation. The overall sponsor's priority is first to select the drug substance based on favourable skin PK and PD parameters, then to select the dose and formulation (drug product) providing favourable skin (/systemic) PK and PD parameters and skin (/systemic) tolerance.

In addition to the psoriasis-related issues, the major limitation of non-clinical models is their lack of predictability to human skin and more particularly to pathological skin, such as psoriatic lesional skin. Besides, from a practical point of view, some of them are not usable for repeated dose (e.g. Reconstructed Human Epidermis (RHE), Franz type diffusion cells); confer limited throughput (e.g. ex-vivo, mini-pigs, humanised mice) and difficulties of supply & facilities (e.g., human psoriatic keratome biopsies for transplantation into immunodeficient mice).

### 1.3.2.1 Pharmacological models of Psoriasis & Perspectives

The available *in-vivo* pharmacological animal models of psoriasis usually exhibit only some features of the disease. Moreover, none of the current models is characterized to the same extent at the cellular and molecular level as human skin diseases. For example, it is not clear whether the same repertoire of leukocyte subsets is represented in both skin types (Petersen, T.K. & Sørensen, P., 2008).

Several approaches have been followed to mimic features of psoriasis in full animals (Danilenko, M.D., 2008):

- (1) The *spontaneous mutations*, which are associated with a more or less psoriasis-like phenotype but not really appropriate to evaluate anti-psoriatic drugs.
- (2) The *genetically engineered* models targeting epidermal keratinocytes, leukocytes or the vascular endothelium (transgenic or knockout rodents).
- (3) The *immune transfer* consists in transferring CD4<sup>+</sup> T-cells into non-transgenic immunodeficient rodents inducing psoriasis-like skin alterations.

Although some of the models reflect some of the psoriasis features (such as acanthosis, altered epidermal differentiation, increased vascularisation, leukocytic and T-cell infiltration), the mechanism of their pathogenesis remain unclear and more importantly, their suitability as true models of human psoriasis appears to be limited. However, several recently described genetically engineered mouse models hold promise for studying many aspects of psoriasis pathogenesis (Danilenko, M.D., 2008).

The (4) *humanisation of immunodeficient mice* appears to be an extremely valuable approach to exhibit psoriatic features in animal models. Humanisation of animals to study psoriasis comprises xenotransplantation of psoriatic (lesional) or uninvolved (non-lesional) keratome biopsies from patients having the disease onto the flank of SCID (Severe Combined Immunodeficiency) mice with or without injection of immune cells (*Figure 19*). The resulting graft survives and maintains the psoriatic phenotype for six to eight weeks (Petersen, T.K. & Sørensen, P., 2008). Xenotransplantation models of psoriasis are valuable tools for the understanding of the molecular mechanisms that underlie the complex interplay in psoriasis but also in drug discovery and development. Although, human psoriatic skin xenotransplantation models are the most biologically relevant to human psoriasis, the difficulties in obtaining human psoriatic skin for transplantation limits the widespread utility of such models (Danilenko, M.D., 2008). Hence, the number of animals that can be studied is limited compared to the other above-mentioned approaches.

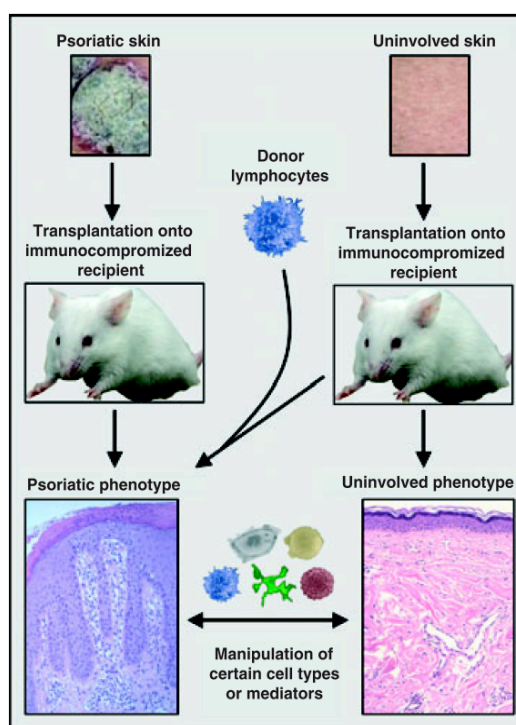


Figure n°19| **Xenotransplantation of human skin onto SCID mice.** Grafts preserve their respective phenotypes following the transplantation. Non-lesional skin, however, can be triggered (by subsets of immune cells) to develop psoriatic features. (Schön, M.P., 2008)

In drug discovery, suitable models must be predictive and reflect human situation as closely as possible, in terms of both positive and negative predictive values. For instance, a compound effective in the model should also be effective in patients, and lack of efficacy in the model should indicate lack of efficacy in patients as well (Boehncke, W.H. & Schön, M.P., 2007). The model must be reproducible, fast, easy to handle and allowing high throughput. Obviously, none of the models presented here fulfils all these characteristics; therefore different models should be used during the process of drug discovery and development. As humanised mouse models are considered a disease-relevant setting, they are therefore *“particularly suited for translational research, namely as an intermediate step between late preclinical research and clinical development, despite their practical limitations”* (Boehncke, W.H. & Schön, M.P., 2007). For instance, humanised models have been validated after the testing of numerous established anti-psoriatic therapeutics showing a robust correlation between the results obtained in the model and those obtained clinically (Table V). Thus, several experimental compounds successfully tested in the psoriasis SCID mouse model are about to enter clinical development.

Therapeutic class	Drug substance	Treatment protocol (SCID)	Outcome in SCID model	Clinical experience
Glucocorticosteroid	Dexamethasone	p.o.	+	+ (A) (rarely used)
	Clobetasol propionate	Topically	+	+ (A)
	Betamethasone dipropionate	Topically	+	+ (A)
Vitamin D	Calcipotriol (Daivonex®)	Topically	+	+ (A)
	1a,25-Dihydroxycholecalciferol	Topically	+	+ (A)
Calcineurin inhibitor	Cyclosporine A	i.p.	+	+ (A)
Antibody	Efalizumab (Raptiva®)	i.p.	+	+ (A)
	Infliximab (Remicade®)	i.p.	+	+ (A)
Cytokine	IL-10	i.p.	-	+ (A)
Experimental drugs	PS519 (proteasome inh.)	i.p.	+	Not reported (B)
	LEO15520	p.o.	+	Not reported (B)

Table V | **Correlation between efficacy of anti-psoriatic drugs in the psoriasis SCID mouse model and clinical experience.** (A): 'established' and (B): 'innovative' (not yet established); i.p.: endoperitoneal. (Adapted from (Boehncke, W.H. & Schön, M.P., 2007; Petersen, T.K. & Sørensen, P., 2008))

As an alternative to *in-vivo* models, reconstituted human epidermal culture models are being developed. Such *in-vitro* models are obtained with normal or psoriatic keratinocytes from human skin put in culture and then treated with a variety of cytokines and/or growth factors. Those models result in reconstituted human epidermal model developing phenotypic characteristics that can mimic psoriatic epidermis (Danilenko, M.D., 2008). This would lead to potential valuable models for drug discovery by modelling cellular responses to penetrating drugs or chemicals (Barker, C.L. & al., 2004) and potentially by identifying biomarkers (e.g. gene expression measurement).

Although mimicking human psoriasis in non-clinical models represents a big challenge in drug discovery and despite the limitations of those non-clinical models, there is great promise in using new and refined disease models in studying psoriasis pathogenesis and in drug discovery & development. Hence, this would confer more confidence in the decision-making in discovery phases and improve candidate selection for First-In-Man (FIM) trials.



### 1.3.2.2 Dermal pharmacokinetics models & Prospects

Once a new compound is conferred a relevant pharmacological effect, pharmacokinetic parameters and bioavailability of the new molecule become essential characteristics for further development. As mentioned in the first section of this thesis, in addition to the PK/bioavailability cause of failure in drug development, pharmacokinetic (PK) parameters could also play a significant role in efficacy, clinical safety and toxicology failures (Garner, R.C. & Lappin, G., 2006). For instance, in *clinical dermal PK*, the first question to be assessed is: *Does the drug substance reach the target site in man?* And if it does, is the concentration high enough to have an effect (efficacy issues) or, does the wrong concentration reaches the wrong target for too long (safety issues). Thus, understanding the dermal PK properties, the bioavailability at the target site and potentially the relation to predictable PD (Pharmacodynamic) or TD (Toxicodynamic) biomarkers, at an early stage, is important to determine whether the product is '*drugable*'. Therefore, such knowledge would enable non-*drugable* compounds to be 'failed' before incurring high resources/costs for later development.

The currently available non-clinical dermal PK models are (1) the *Franz's* cell model using *ex-vivo* human skin, (2) *In-vitro* reconstituted human skin and (3) *In-vivo* rodents and non-rodents (mini-pigs) (*cf Table 4*).

#### *Franz's cell model*

Firstly developed by *Franz, T.* thirty years ago, the *Franz's* cell model is a diffusion cell in which *ex-vivo* excised human skin is placed. The human skin slices come from human donors after surgical procedures. This technique consists in measuring the percutaneous absorption of drugs *in-vitro* (Guideline n°428, OECD, 2004) in order to model clinical situations for the dermal absorption of drugs. After one single dose (finite dose similar to what is used in a clinical setting) and 16-hours of incubation, drug concentrations can be measured separately with LC-MS/MS or HPLC analysis in the following compartments: in the excess (non absorbed dose), the stratum corneum, the epidermis, the dermis and in the receptor fluid (absorbed dose) as well (*Figure 20*). Such *in-vitro* percutaneous absorption studies, also called liberation-penetration studies, are routinely performed in early development as a screening tool to select the optimum formulation composition, as well as the



appropriate concentration of the active substance in the formulation, all to ultimately achieve the desired magnitude, profile, and duration of dermal absorption.

Perspectives for this surrogate model are (1) to compare the *in-vitro* skin distribution with the one found in clinical trials, obtained under similar conditions (2) to combine penetration values to biological activity data (PK/PD), (3) to use psoriatic skin rather than healthy *ex-vivo* skin and (4) to refine the model in order to perform repeated dose (instead of a single dose) in order to get concentration time profiles.

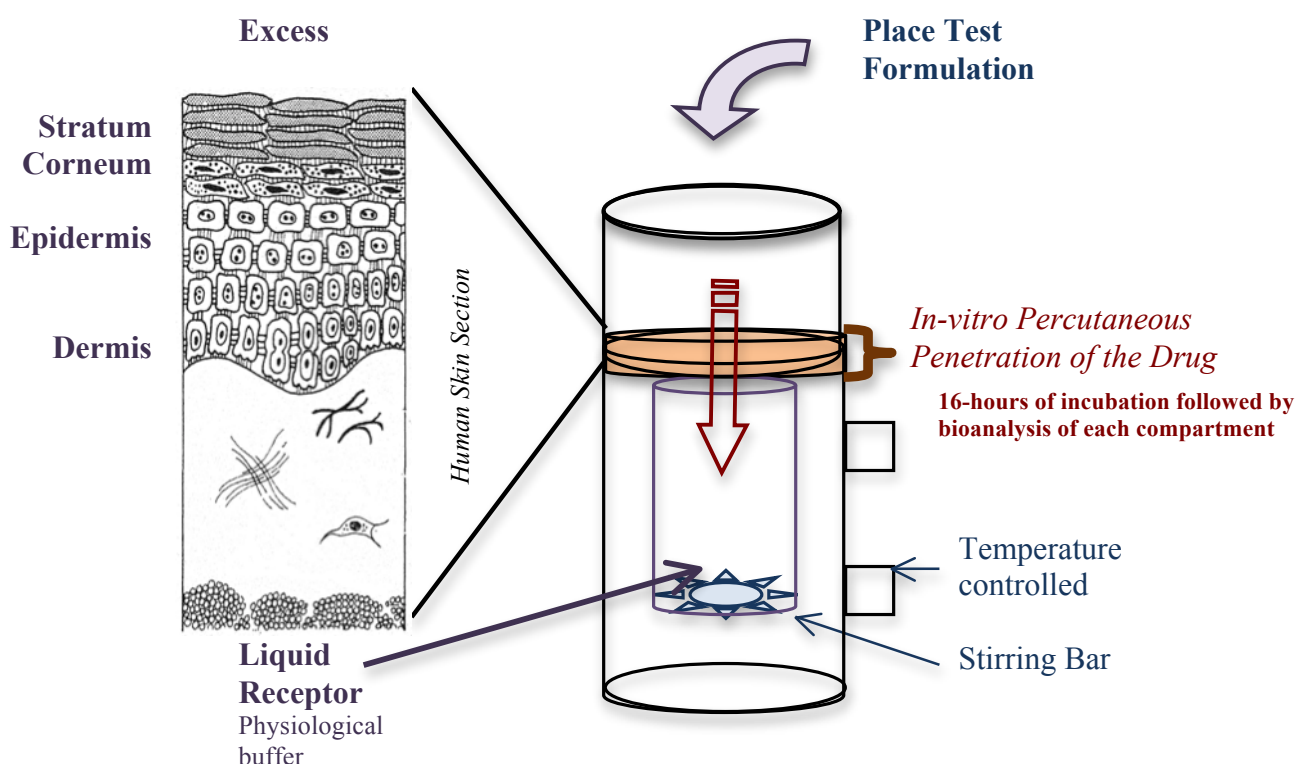


Figure n°20 | **Franz type diffusion cell model using human skin.** Adapted from Conrex Pharmaceuticals website & Galderma documentation

### *In-vitro reconstituted human skin or epidermis (RHE) model*

RHE are cultured skin models obtained from healthy or psoriatic keratinocytes. Such model can be used as a skin delivery model to evaluate the absorption and penetration of a formulated compound as well as its skin metabolism. A perspective of such model would be its use in *Franz cells* in countries where cultural roadblocks preclude the use of natural human skin. Meanwhile, a normal stratum

corneum and competent barrier function must be developed. They can also be used as pharmacological models (cf *section 1.3.2.1.*).

The *ex-vivo* model, compared to the RHE model, is currently more valuable in predicting topical delivery, notably by its use of human materials.

#### Mini-Pigs model

Nowadays in dermatology, the animal of choice for non-clinical studies is the mini-pig, which is the closest animal to human in terms of skin features. Moreover, Competent Authorities recognize it. However, this model may not be as predictive to human as we wish it is. For example, in a recent study performed in Galderma, two formulations (ointment 0.1% of a compound A and lipophilic gel with 0.9% of the same compound) were tested both in mini-pigs and in clinic. In mini-pigs, the cumulative irritation was higher with the gel than with the ointment. In human, the results showed a low efficacy with the ointment 0.1% and neither efficacy, nor local side effects with the lipophilic gel. It was supposed that the lipophilic formulation would inhibit the penetration of the active substance in humans but not in mini-pigs. However, at this time, they did not have penetration data supporting that hypothesis. This *formulation effect* was therefore not predictable from the conducted study. This example showed that the mini-pig model was not completely predictive of the clinical situation with this compound, in psoriasis.

PK behaviours of new drugs in humans are usually determined by allometric scaling which considers a number of “*anatomical, physiological and biochemical variables*” in mammals (such as tissue volume, blood flow and process rate) and scaled based on body weight. Conversely, *physiology based-PK models* describe the tissues in the body as a system of tissue compartments, interconnected via the vasculature (Poggesi, I., 2004). The lack of such models may explain the lack of precision and accuracy of PK predictions of the underlying ADME behaviour in humans. Although the mini-pig seems to be the most relevant animal mimicking human skin, the example above shows its limits. Thereby robust *in-vitro*, *ex-vivo* models or humanised animals must be optimised thanks to currently available technologies. In addition, the suitability of the PK measuring methods is essential in providing relevant results.

The objective is to (1) evaluate the suitability of all dermal evaluation tools, (2) refine non-clinical models (including their good characterisation) in order to consolidate their predictability to human and finally to (3) validate the models as well as the tools by correlating non-clinical to clinical results. Further perspectives to this research are to combine PK parameters and PD biomarkers predicting human efficacy or toxicodynamic biomarkers predicting human safety. Such improvement would enhance the relevance and consistency of drug discovery & development and address some of the challenges faced by dermatological pharmaceutical industries (*cf Litterature review 1.1*). However, such advances underline long-term investment and require time and resources before seeing any benefits.

### **1.3.2.3 Safe First-In-Man starting dose & Systemic toxicity & local tolerance**

The estimated safe human starting dose in FIM trials (*mg/kg/day*) is determined according to the drug product characteristics and the *in-vitro* predicted penetration data (e.g. *in-vitro* liberation-penetration studies). Safety margins for FIM trials are determined according to a ratio between the Human Equivalent Dose (HED) and the human dose (FDA, 2005). The HED itself is based on the Non-Observable Adverse Effect Level (NOAEL) from the most sensitive animal species, applying an allometric correction factor. Lastly, the human dose is based on liberation-penetration data and the average body weight of the study subjects.

Systemic safety prerequisite before FIM is pretty well described in regulatory guidelines. However a number of questions remain unsolved: *is the most sensitive animal species the most relevant to human toxicity?* (e.g. is cardiotoxicity found in dogs really predictable to human potential cardiotoxicity?). Although some *in-vitro* testings exist to detect systemic toxicities (i.e. assessment of stability in microsomes and hepatocytes...), there are few tests available to assess cutaneous tolerance (e.g. mouse ear oedema, small zones application on mini-pig models, rabbits...). Both appear to be more or less predictable of human local tolerance depending on the tested compound. One of the current objectives is to determine biomarkers of systemic and/or local toxicity. The only ones recognised today by the regulatory authorities are biomarkers of renal function that can predict nephrotoxicity (e.g. cystatin-C, Albumin, KIM-1,  $\beta$ 2-microglobulin...) (Muller, P.Y. & Dieterle, F., 2009).

## 1.4 Early clinical trials in psoriasis

As seen in *section 1.1.1*, Pharmaceutical companies face growing pressure to improve their R&D productivity, to get new drugs on market sooner, and to develop medicines with demonstrable advantages over current therapies. For instance, firms and Regulatory Authorities have recognised that the drug development process needs optimisation for efficiency in view of the return on investments (Dingemanse, J. & Appel-Dingemanse, S., 2007). Such optimisation starts at the interface between non-clinical research and Early Clinical Evaluation (ECE), which is made efficient thanks to the full integration of preclinical and clinical research activities: Patient driven process – Humanisation of research. With this in mind, clinical protocol designs evolve toward increased protocol innovation & complexity in order to learn more from Phase I trials, reduce trial costs and speed development cycles. Greater use of biomarkers and information technology, improved protocol designs, better recruitment and retention of volunteers are helping today (Kaitin, I.K. & al., 2010). The overall objective is, at the end of the day, to terminate more unpromising drugs earlier and to avoid wasting time, energy and money.

### 1.4.1 Streamlining of clinical research

Traditionally, Clinical Trials (CTs) were conducted with an empirical manner, that is, designed to assess whether patients have shown improvement or had adverse reactions, rather than to explore the underlying physiological mechanisms of product performance (Topic 2, FDA, 2006). This was due to a dearth of knowledge and evaluative tools for exploring pharmacologic mechanism and pharmacokinetic profiles. Moreover such trials could answer only a few questions within a single trial leaving unanswered questions (Topic 2, FDA, 2006).

Such awareness, alongside scientific and technological progresses, has led to restructuration of much pharmaceutical companies by introducing Early Development Units which stand in-between research and development. Within *Galderma*, the Early Development department encompasses various teams, including the Early Clinical Evaluation group (formed by the Clinical Research and Cutis<sup>12</sup>) working together with

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<sup>12</sup> CUTIS : Clinical Unit for Testing and Investigation of Skin

early formulation, early ADMET as well as competitive & scientific intelligence, intellectual property, and licensing units. Its overall goal is to fill the pipeline with new projects and be selective at a much earlier stage in the research and development process to ensure that only optimal candidates will enter full development. *Figure 21* shows the Go/ No-Go decision process for a new drug, from target identification to marketing approval application. From selection of a candidate to the release of a drug product for full development, early development is associated with clinical knowledge acquisition.

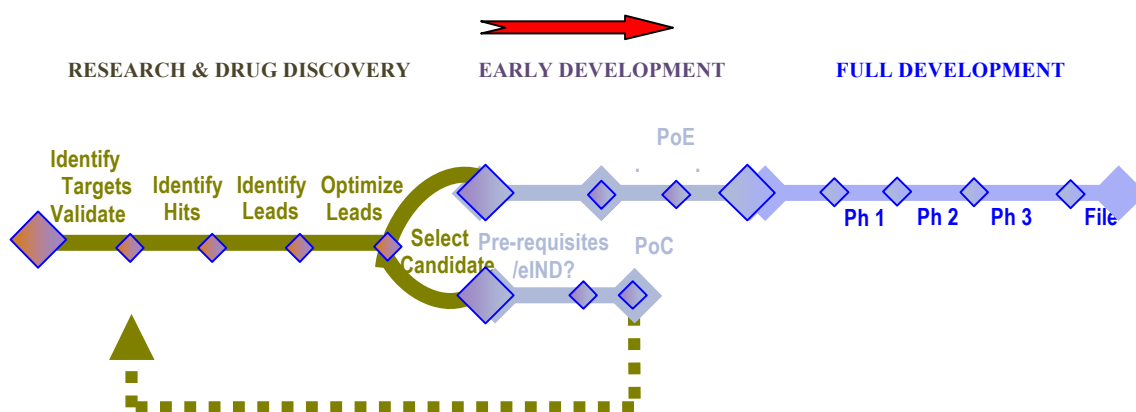


Figure n°21 | **Decision making throughout drug discovery and development process.** (Adapted from Galderma documentation).

The quality of the Go/ No-go decision process in early clinical development may be improved with reliable information on safety, efficacy dose range and exposure-response characteristics. The translational medicine concept goes toward this direction with the collaboration between early clinical development and research. For instance, the development of new tools help elucidate the causal mechanisms underlying product safety and efficacy, in order to bridge the gap between the early stages of the development of a new drug and its potential effects in humans.

#### 1.4.2 Innovation in clinical trial designs & Novel screening methodologies in psoriasis

In opposition to empirical trials, early clinical researchers perform *learning trials* in order to gain as much knowledge as possible in a short period of time (e.g. 4

weeks) on few subjects (between 20-30 subjects). The exploratory drug development's strategy consists notably in validating new clinical pharmacological models, new clinical endpoints. Also, new evaluation tools are developed, and then integrated in exploratory clinical trials, to test and compare candidates.

#### 1.4.2.1 Innovation in clinical trial strategies

✿ **Traditional First-In-Man (FIM)** studies usually involve testing a single drug product candidate, selected after extensive preclinical testing; are conducted in healthy volunteers; with single followed by multiple ascending dose (SAD & MAD) design; and standard preclinical toxicology package (including repeated-dose toxicity studies of at least 2 weeks duration in two species). However, the low success rate with the conventional clinical development pathway (*cf FDA's Critical path initiative, 2004*) led to changes in early-stage clinical research toward new recommendations and guidelines on exploratory clinical trials designs and the necessary use of biomarkers. Such trials would be performed before the traditional phase I package in order to decide earlier whether the project is viable for development.

Besides, the *TeGenero TGN1412* clinical trial accident in 2006 has led to a new guideline on the determination of the starting dose for high-risk investigational products (EMA, 2007).

✿ Indeed, **exploratory clinical studies** (or eTrials/ eIND) “*are intended to be conducted early in Phase I studies, involve limited human exposure, have no therapeutic or diagnostic intent, and are not intended to examine maximum tolerated dose*” (ICH M3 (R2), 2009). For instance, the general principles of exploratory clinical trials are: (1) limited number of subjects, limited dose range and limited duration, (2) early identification of promising candidates for further development (and eliminate candidates lacking promise), (3) reduction of the number of subjects and resources (including amount of drug) needed to identify promising drugs, (4) potential risk to human subjects is lower than for a traditional phase I study, (5) less (or different) preclinical studies than required for traditional first in man studies. The following *table VI* summarises the three different guidelines currently available that tackle exploratory clinical trials. The five different approaches described by the ICH guideline in 2009 will not be described in details since it is not in the scope of this thesis.

Although these approaches allow limited non-clinical testing programs (e.g. limited safety pharmacology), the non-clinical safety package will have to be completed before further clinical trials.

	<b>EMA (2004)</b> "Position Paper on Non-Clinical Safety Studies to Support Clinical Trials with a Single Microdose"	<b>FDA (2006)</b> "Exploratory IND Studies"	<b>ICH (2009)</b> "Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals" (ICH M3(R2))
Duration	Single dose only	Up to 7 days	Up to 14 days
Approaches	Microdose study only (100µg or 1/100th of pharmacological dose)	- Microdose study - Pharmacological, but not toxic - Dose escalation permitted in pharmacological range	- Limited Microdose study - Extended microdose study - Single pharmacological or therapeutic dose - Repeated-dose study up to 14 days, therapeutic dose range (2 approaches with slightly different preclinical requirements)
Endpoints	<ul style="list-style-type: none"> <li>• Pharmacokinetics/ distribution</li> <li>• Pharmacodynamics</li> <li>• Comparison of multiple candidates</li> </ul>		

Table VI | **Summary of the exploratory clinical trials guidelines.** (Adapted from EMA, 2004; FDA, 2006 and ICH (2009))

✱ In dermatology, according to the level of biopharmaceutical and pharmacologic knowledge of the compound, early **Proof-of-Concept (PoC)** and **Proof-of-Efficacy (PoE)** experimental studies (*Figure 21*) can be registered as FIM Phase I trials, as long as the principal objective of the trial is safety. They are conducted as early as possible in order to evaluate the value of a drug in clinic, in the pathology of interest, before going further in development.

*PoC studies* in dermatology correspond (for example) to the first clinical evaluation of an investigational drug for which a new formulation has been developed and for which there is no proof of clinical efficacy of the compound in the indication of interest (with this formulation). The objective of such studies is to clinically evaluate a new therapeutic class on a disease and give early readouts on the activity profile, the cutaneous exposition profile, and local tolerance data. Such information will facilitate formulation selection and more importantly, evaluate the viability of the project in this selected indication. According to the results, the formulation or even the drug substance may be refined.



*PoE studies* are performed when there is already a recognised efficacy in the indication of interest with another drug from the same therapeutic class (e.g. vitamin D derivatives in psoriasis) or, if one or several formulations of another drug from the same therapeutic class already exist in another indication (e.g. retinoids tested for acne then on psoriasis). The selection criteria are then to show that the activity profile is at least similar to the other drugs of the same class, that the cutaneous profile and the drug concentration are optimal, that the drug confers a good local tolerance and finally, that the compound is well adapted to the pathology of interest. The design of such studies in psoriasis will be detailed below.

#### 1.4.2.2 Innovative clinical trial designs in dermatology and particularly in psoriasis.

For experimental clinical trials in psoriasis, the psoriasis *small plaque bioassay* or *mini-zone test*, derived from the KJ Dumas and JR Scholtz method (Dumas, K.J. & Scholtz, J.R., 1972), is one of the most widely applied assays (Katz, & al., 2000). By definition, these trials, although classified as Phase I, are conducted in psoriatic patients. This assay was first developed for the screening of GCs but was then adapted for the screening of other anti-psoriatic compounds. In addition to screening for anti-psoriatic effects, *Dumas-Scholtz plaque test* can help answer other questions, such as



Figure n°22 | Dumas-Scholtz plaque test on a psoriatic patient's forearm.

frequency of use, dose relationship and comparison between several formulations (Petersen, T.K. & Sørensen, P., 2008). The psoriasis mini-zone plaque test is relatively simple, with very low systemic exposure and a duration of 4 weeks. An essential advantage in dermatological CTs is the simultaneous topical treatment of multiple active compounds and controls on the same subject to clear inter-individual variability, especially with the *Dumas-Scholtz plaque test* where several sites of application can be



localised on the same psoriatic lesion (*Figure 22*). This (usually) randomised, within-subject design avoids inter-individual variability and therefore increases the study power since a dose range of the active compounds, the vehicle, as well as reference compounds may be applied on the same psoriatic patient. Therefore, such studies require fewer recruited subjects to draw statistically significant conclusions, compared to parallel design studies. Corticoids and vitamin D derivatives are often chosen to be the positive comparators as they are efficient in the treatment of psoriasis. The evaluation of the study products will be done thanks to efficacy and safety scoring. For instance, the Total Sum Score (TSS) including erythema, induration and scaling evaluation and the clearing score are used for clinical evaluation of the severity and irritation scoring for local safety evaluation.

As seen on *Figure 22*, for each subject, test sites of usually 2-cm diameter are selected on a predetermined lesion, and delineated with a circular adhesive device. After the applications, tests sites may be covered occlusively (using Finn Chambers), or non-occlusively (with non-occlusive gauze), according to the drug tested and the aim of the clinical trial. The occluded approach favours the penetration of the compound into the skin leading to increased efficacy (*e.g.* GCs) but also to increased irritation potential, compared to 'normal' clinical usage. The non-occluded approach is closer to the current clinical practice.

In PoC studies, the occluded approach may be chosen if the local tolerance is good, to ensure that the drug substance reaches the target site and therefore really assess whether the new compound confers a real benefit or not. Indeed, information on the dermal permeation of a compound is essential to assess the efficacy or the non-efficacy of a new compound. The *in-vitro* liberation-penetration study using *ex-vivo* healthy human skin aims at measuring dermal penetration of new compounds in order to select the formulation that confers the most favourable penetration of the drug substance.

One may ask whether generated data from liberation-penetration studies (performed with non-viable human skin) do reflect the situation found in clinic on human viable skin including its skin metabolism and its vasculature for instance. Besides, can we expect such *in-vitro* model to predict permeation of human psoriatic

skin as well? Before answering that question, the necessary first step is to assess the impact of psoriasis disease on the penetration of a topical agent applied on the skin surface. These questions will be addressed in the results and discussion of this thesis.

As introduced throughout this section, the objective of early experimental clinical trials or PoC studies is to conduct more integrated studies to examine multiple clinical issues and gain as much knowledge as possible on a compound in a specific indication. For instance, for a FIM experimental PoC study, the first objective will necessarily be the safety and the second can be the efficacy assessment. Other objectives would consist in the collection of complementary information through new evaluation tools, including recently developed sampling and analysis technologies. Indeed, new evaluation tools may (1) provide early PD information in human based on biochemical or imaging biomarkers, surrogate endpoints or clinical endpoints (*Table VII*), (2) provide early PK profile in human (e.g. skin concentration-time profiles using dermal microdialysis), (3) measure skin distribution using different technologies (e.g. skin biopsy, tape-strippings, microdialysis, etc) and finally (4) validate non-clinical models by comparing clinical and non-clinical results. For instance, a perspective in experimental clinical trials would be to develop maybe more focused early PK, PD or PK/ PD studies (matching with the eTrials and ICH guidelines) that would require limited pre-requisites and would provide early robust information for improving the decision making in the screening of topical NCE in early drug development in psoriasis.

<b>Biomarker</b>	A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers may be: <ul style="list-style-type: none"> <li>- Disease-related by describing the disease pathology and used to determine if the drug has the desirable effect on the disease</li> <li>- Target-related, used to determine if the drug has the desirable effect on the target.</li> </ul>
<b>Clinical endpoint</b>	A characteristic or variable that reflects how patient feels, functions, or survives.
<b>Surrogate endpoint</b>	A biomarker intended to substitute for a clinical endpoint.

Table VII | **Definitions of the different type of biomarkers.** (*Biomarkers Definition Working Group (2001); Petersen, T.K. & Sørensen, P., 2008*)

### 1.4.2.3 New tools to assess human PK and/ or PD skin parameters

As blood sampling does not reflect the skin, new evaluation tools have been developed including refined sampling methods. Bioanalyses are then performed by conventional analytical methods (e.g. histology, immunohistochemistry, HPLC...), 'omics' high-throughput methods (e.g. Genomics, transcriptomics, proteomics, metabolomics...) or advanced imaging techniques. The following table presents the type of clinical samples, the sampling methods as well as the type of information that can be collected in psoriatic patients in clinical trials (*Table VIII*).

Type of clinical sample	Sampling methods	Type of information provided (exploratory data)
Skin biopsies	Punch skin biopsy	PD/ TD biomarkers research <sup>1</sup> Skin distribution/ Bioavailability
Squames/ Stratum corneum (SC)	Tape-Stripping	PD biomarkers research Skin distribution? (high variability, usually not target site) Bioequivalence
Scalp hair follicle (scalp psoriasis)	Plucking of scalp hair follicles	PD/ TD biomarkers (e.g. gene expression profiling <sup>2</sup> ) PK/ PD profile
Microdialysis harvests	Microdialysis/ Open Flow microperfusion	PD / TD biomarkers research PK: Concentration-time profiles of the active molecule/ Bioavailability/ Bioequivalence PK/ PD profile
Photographs	3D Digital Imaging <sup>3</sup>	Surrogate endpoints

Table VIII | **Different evaluation tools available in psoriasis early clinical development.** <sup>1</sup>Kvist, P.H., 2009; <sup>2</sup>Aubert, J., 2010; <sup>3</sup>Fadzil, M.H.A. & al., 2010.

The objective of dermal PK methods is to measure the topical skin bioavailable drug's concentration at the target site and relate to PD biomarkers or clinical efficacy. Once again, there is no single method that provides all PK parameters needed to estimate clinical topical bioavailability. However, the available options are complementary and, combining several of them should, theoretically, give appropriate information on the PK parameters of a new compound. The different experimental methods that can assess drug penetration into and/or across the skin are characterised in the table presented below (*Table IX*). The *in-vitro* method (*Diffusion cell*) has been detailed in the PK model section (*cf 1.3.2.2*). The currently most promising *in-vivo* methods that can be used on psoriasis skin (i.e. *dermal microdialysis*, *skin biopsies* and *Tape-stripping*) will be detailed below. *Confocal Raman* and *skin blisters* have not shown yet to be valuable tools in dermal PK measurement.

	Method	Measurement site	Type of PK information	Temporal resolution (concentration time profile)	Technical simplicity
<i>In vitro</i>	Diffusion cell (Franz cell)	Transport in the across skin	Skin distribution	+/-	+
<i>In vivo</i> : non or minimally invasive	Tape stripping	Stratum corneum	SC concentration	++	+
	Confocal Raman	Stratum corneum (+/- epidermis)	SC concentration Epidermis(?)	+	+
	Dermal microdialysis	Dermis	Bioavailability (free molecule)	++	-
<i>In vivo</i> : invasive	Skin Biopsy	Epidermis/ Dermis	Skin distribution: Epidermis / Dermis concentration	0	+/-
	Skin blister	Dermis	Dermis concentration(?)	0	+/-

Table IX| **Different available methods to assess drug penetration into and/or across the skin.** SC: Stratum Corneum. (Herkenne, C., 2008; Galderma internal documentation).

The particularly interesting value of such *in-vivo* methods is that they can be used similarly on human subjects as well as on animals and therefore make comparison possible. Moreover, human samples can also be compared to the PK, PD or PK/ PD *in-vitro* and *in-vitro* data.

#### ✳ **Dermal microdialysis technique**

Dermal Microdialysis (DMD) technique has been introduced in the last decade in studies measuring dermal drug concentration in the living tissue after topical administration. It was shown to be a promising tool for the assessment of topical drug delivery (Schnetz, E. & Fartasch, M., 2001). The method is based on the passive diffusion of compounds (concentration gradient) across an ultrathin, semi-permeable hollow fibre structure. This probe is implanted superficially into the dermis, parallel to the skin surface. It is typically a few tenths of a millimetre in diameter and represents, in theory, a permeable blood vessel perfused with physiological solution, which equilibrates with the extracellular fluid of the surrounding tissue (*Figure 23*, Benfeldt, E. & al., 2007). The exchange of material is driven by passive diffusion, allowing exchange of small, diffusible molecule such as unbound pharmacologically active substance present in the intercellular fluid. Considering the dermis as the target tissue, the interstitial fluid is regarded as the true anatomic effect compartment. An essential advantage of DMD is its ability to continuously monitor the extracellular concentration of a drug. It provides a “*pharmacokinetic profile, with high temporal resolution, of the drug in the target tissue*” (Herkenne, C. & al., 2007). For instance,

the dialysate can be pooled every 3-4 hours during 24h. Moreover, as this technique is claimed to be minimally invasive, several sampling sites can be studied simultaneously in the same volunteer.

This method has demonstrated its feasibility in animals as well as in humans. It was shown to be useful *“to assess free concentration in the dermis or subcutaneous tissues, to compare different formulation: bioavailability/ Bioequivalence and finally to measure whether therapeutic concentration are reached in the skin”* (Herkenne, C. & al., 2007).

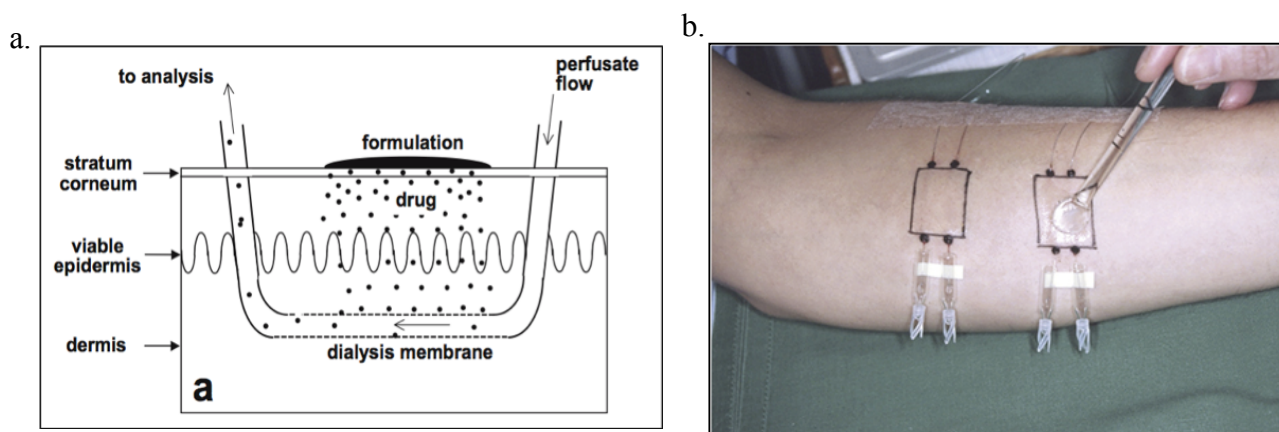


Figure n°23| a. **Schematic of the most prevalent design of Dermal Microdialysis.** The free active substance (black dot) in the interstitial fluid diffuses across the dialysis membrane to the perfusate flow (physiological solution) and is then collected in the dialysate for bioanalysis. (Herkenne, C. & al., 2007). b. **Dermal Microdialysis performed on a subject.** (Benfeldt, E. & al., 2007)

Whereas, DMD can only measure small, unbound molecules, another tool has been developed to overcome limitations of the state of the art methods. In contrast to the dermal microdialysis, the Open Flow Microdialysis (OFM) has a more porous membrane. In addition to the free active molecules, OFM can collect plasmatic proteins conjugated molecules, lipophilic molecules as well as large molecules, in the dialysate. The OFM has once been used in a PK/ PD study involving psoriatic patients (Bodenlenz, M. & al., 2009).

Such methods are therefore promising not only for PK measurements but also for PD biomarkers research. Also, these techniques will help understanding the impact of psoriasis on drug cutaneous PK profiles compared to healthy skin. They are of great promise in discovery and development of new anti-psoriatic drugs.

### ✧ *Tape stripping*

The tape-stripping technique is a Dermatopharmacokinetic (DPK) method that was described by Shah, V.P. in 2001. The procedure consists in performing repeated applications and removal of adhesive tape on the skin surface in order to harvest consecutive layers of stratum corneum (SC), the outermost layers of the skin (*Figure 24*). The strips are then pooled (usually 2 by 2; the first one is discarded) and analysed by High Performance Liquid Chromatography - mass spectrometry (HPLC-MS) for example. The results give information about cutaneous drug or excipient levels in the different layers of the stratum corneum after topical dermatological treatment. For instance, after an analysis of each strip, it was shown that the *“drug concentration in the SC decreases log-linearly, and that about 90% of the concentration is found in the first 10 tape-strips”* (Benfeldt, E. & al. 2007).

This technique is relatively non-invasive and painless, given that only corneocytes embedded in their lipid matrix are removed. Even though the stripping remains an external insult, the barrier function of the SC is rapidly restored thanks to a homeostatic repair response in the epidermis (Herkenne, C., 2008). However, on pathological skin, such as psoriatic skin, it may lead to disease worsening (*Koebner phenomenon*<sup>13</sup>).

Tape stripping is a useful technique to assess the local bioavailability of drugs or compare several drugs whose target site is the SC itself (such as antifungal agents, UVA/UVB filters or antiseptics) (Herkenne, C., 2008). As the SC barrier is usually the principal resistance to the penetration of topically dermatological agents, it was argued that drug levels in the SC should be correlated with those attained in the epidermis and/ or the dermis (Herkenne, C., 2008). Using the tape-stripping technique may be relatively interesting to collect preliminary information since it is less invasive than dermal microdialysis or skin punch biopsy, which are techniques that measure the concentration of a topically applied drug in the underlying viable layers of the skin.

However, tape stripping has several limits, the main one being the lack of standardised procedures (Shah, V.P., 2005). This is why, in May 2002, the FDA Draft

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<sup>13</sup> **The Koebner phenomenon** can be obtained by repeatedly applying and removing a piece of tape from the individual's skin (tape stripping), which affectively disrupt the skin barrier and leads to inflammation.

Guidance Document on “*Pharmacokinetics Applied to Drug Concentration Measurements in stratum corneum (SC)*” was withdrawn. Indeed, there was a lack of correlation between the drug concentration in SC (determined by tape-stripping) and clinical efficacy but also, DPK results were different from one lab to another (Benfeldt, E. & al. 2007). The tape-stripping procedure confers a high coefficient of variability that may be explained, at least in part, by the lack of standardized procedures (e.g. pressure applied on the skin, the removal of the strip, the type of adhesive, the prevention of drug degradation by the extraction process... etc).

One advantage is the possibility to easily implement tape stripping either in human, *in-vivo* (e.g. in mini-pig) or *in-vitro* (on excised human skin) to evaluate local bioavailability and compare the results between different models.

The possible use of this method is to compare formulations, to evaluate the effect of a vehicle or enhancers, to predict diffusion profiles from short duration exposure, as well as to assess bioequivalence. Moreover, this method was recently used for PD evaluation (biomarkers) in the scales, thanks to proteomic and transcriptomics analysis.

#### ✧ **Skin punch biopsy**

Skin biopsies received attention only recently. Indeed, the use of skin biopsies “*for tissue sampling and analysis post-drug application have not attracted much attention as a routine approach for use in vivo*” (Herkenne, C., 2008). Although few years ago skin biopsy was intended to be restricted to animal and *in-vitro* studies because of its aggressive nature, it starts now to be used in clinical trials notably for pharmacodynamic biomarkers measurement (Kvist, P.H. & al., 2009; Phillips, R.L., 2005). Skin biopsies are more difficult to obtain than blood. As punch biopsies are invasive and consequently painful, they must be performed under local anaesthesia. Tolerability is therefore an important factor that may affect the recruitment of subjects who undergo a biopsy procedure in a clinical trial. Some specific exclusion criteria related to the biopsy must be added to the protocol (e.g. allergy to local anaesthetics, history of coagulation trouble or abnormal healing...).

However, skin biopsies have several distinct advantages. Punch biopsies normally penetrate completely through the epidermal and dermal layers and have a 2- to 4-mm diameter (*Figure 24*). After resection, the epidermis and dermis can be divided. After processing profiling, skin biopsies may be used for several purposes such as pharmacodynamics, early evidence of adverse effects or efficacy (Phillips, R.L., 2005) and pharmacokinetics. Skin biopsies have the advantage of offering a “snapshot” of drug distribution in the different skin layers, which is very interesting when the objective is to get information on the bioavailability of a topical agent in its target site. This was therefore the chosen technique to be used in the clinical trials that will be presented in the next chapters.

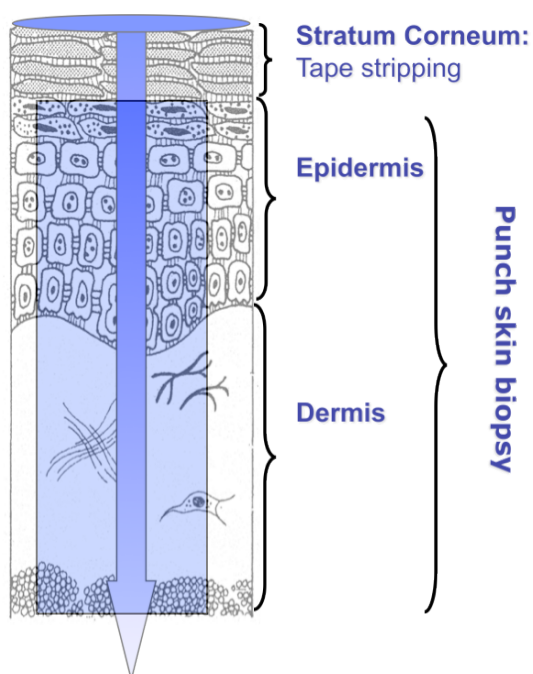


Figure n°24 | **Skin punch biopsy.** It penetrates completely through the epidermal and dermal layers and have a 2- to 4-mm diameter. A skin punch biopsy is normally performed after tape stripping in order to remove the SC. *Source: adapted from internal Galderma documentation*

In opposition to blood sampling or tape-stripping, one main limit of techniques such as dermal microdialysis and skin punch biopsies is that they cannot be performed regularly during a clinical trial, and that they may impact study recruitment. However, both are appropriate methods for collecting PD, PK (and PK/ PD for the microdialysis) information after a topically applied product.



## Chapter 2: AIMS & OBJECTIVES –

### 2.1 Hypothesis

As traditional strategies in drug development have major limitations (*Literature review section 1.1*), new approaches in early development for psoriasis must:

- Be more disease-specific (i.e. based on the pathophysiological hallmarks of the disease) (*Literature review section 1.2*)
- Rely on relevant non-clinical models (*Literature review section 1.3*) and
- Introduce human as soon as possible since the best model remains the human model (*Literature review section 1.4.*).

The clinical hypothesis of the skin distribution cross analysis is the following:

*Molecule A* comes from a well-established oral drug registered for a non-dermatological disease. Its pharmacological properties were found to be potentially useful for the treatment of psoriasis. It was then reformulated (as a cream formulation) in order to allow topical administration.

Within the *Drug A* formulation development, one *In-vitro* liberation-penetration study was conducted using human excised skin in *Franz* cells. This study showed the skin distribution of molecule *A* in the different layers of the non-viable skin. However, such data on *Drug A* skin distribution were not available in viable human skin.

The translational approach here consisted in measuring the concentration of *Drug A* in the different layers of the skin in clinical settings (i.e. healthy volunteers and psoriatic patients) thanks to recently developed sampling tools (i.e. tape stripping followed by skin biopsy). A cross analysis was then carried out in order to compare the following results:

1. Skin distribution data on healthy skin (healthy volunteers) *versus* Clinical study on psoriatic skin (patients)

2. Overall cross analysis, comparing liberation-penetration *in-vitro* study on excised healthy human skin *versus* Clinical studies performed on healthy volunteers and psoriatic patients

The comparison of such experimental results allowed exploring:

- Firstly, the impact of psoriasis pathology on *Drug A* skin penetration compared to healthy skin in clinical settings. This would help us to know whether skin distribution results from healthy volunteers reflect what is happening in psoriatic patients, notably for this specific molecule in this specific formulation.
- Secondly, the ability of *in-vitro* liberation-penetration studies to predict whether or not the drug substance reaches the target site (Epidermis + Dermis in psoriasis) in human and at what concentration (non-viable skin *versus* viable skin). If a link does exist between the *Drug A* concentrations *in-vitro* and *in-vivo*, the translation (liberation-penetration studies → clinical studies) would be validated, for this molecule in this formulation. Such *in-vitro* studies could, one day, be an alternative to the invasive skin biopsy procedure performed in clinic by predicting drug concentration at the target site. If so, negative results of an early clinical trial associated with *in-vitro* data (showing that the drug was actually present at the target site at a potentially effective concentration) would conclude that the drug is not effective in the studied indication. The early No-Go decision would therefore be facilitated.

## 2.2 Aims

This thesis' aims, based on the results of three studies testing *Drug A* and following the above-introduced translational approach, are:

1. To present and analyse skin distribution results (experimental objectives) of three different trials with different designs.
2. To perform a *Drug A* skin distribution *cross analysis*, comparing *in-vitro* and clinical data in order to evaluate:

- The difference in the penetration profile depending on the dosage of *Drug A* and the type of skin (healthy human skin *versus* psoriatic human skin) in order to assess the impact of psoriatic skin on the permeation of *Drug A*, compared to healthy skin, in clinic.
  - The ability of the *in-vitro* liberation-penetration results after a single application to predict the skin distribution and concentration of *Drug A* in human clinical settings (healthy human skin and/ or human psoriatic skin) after repeated applications.
3. To evaluate the benefit of conducting such exploratory studies in early clinical development: Are the results obtained with tape-stripping and skin biopsy robust enough to support Go/NoGo decisions and therefore benefit early screening?

## 2.3 Objectives

In order to achieve these aims, two clinical studies and one *in-vitro* study have been conducted:

- In clinical settings (healthy volunteers and psoriatic patients) of which exploratory objectives were the measurements of the concentration of the active molecule (*Drug A substance*) in the different skin layers. The measurements were performed 24-hours after the last applications of a three week study, using tape-stripping and skin biopsies techniques in subjects who agreed to undergo the procedure.
- *In-vitro* study called “liberation-penetration study” performed on excised human skin in *Franz* cells, carried out earlier in development, also measured the skin distribution of the *Drug A* substance as well.

The following figure schematises the three studies that will be compared (*Figure 25*).

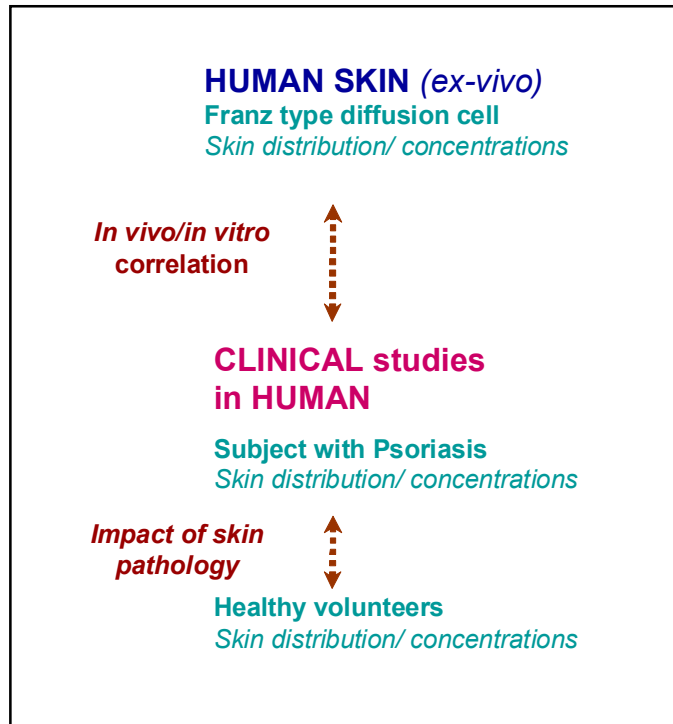


Figure n°25 | Cutaneous measurements (skin distribution/ concentrations) in different models.  
(Adapted from Galderma documentation)

## Chapter 3: MATERIALS & METHODS –

The following chapter contains two distinct sections:

- the first describes the work carried out for the literature review.
- the second details the materials and methods that were used for the setting of the clinical trials and the *in-vitro* study testing *Drug A*.

### 3.1 Materials & Methods used for the literature review

The two questions introduced in the literature review were:

- *What are the criteria to be taken into consideration for topical anti-psoriasis new drug discovery & development success?*
- *How Translational Medicine can contribute to the improvement of the early screening of new topical treatments for psoriasis?*

Different materials were used for answering these questions. First, bibliographical search was carried out using the Galderma documentation research setting called *SICI* (*scientific Information & Competitive intelligence*), as well as on *PubMed*. The main key words were: “translational medicine”, “experimental medicine”, “exploratory clinical trials”, “dermatology”, “psoriasis”, “non-clinical models”, “pharmacokinetics”, “bioavailability”, “pharmacodynamics”, “biomarkers”, “strategies”, “and modelling”, “skin biopsy”, “dermal microdialysis”, “tape-stripping”, “Dumas Scholtz”, “plaque test”, “mini-zone”, “phase I”.

I completed this research with Galderma internal documentation to which I had access, with testimonies from colleagues & internal experts, with congress reports as well as with my Translational Medicine coursework and assignments. Moreover the following EMEA, FDA or ICH guidelines helped me, including:

- “*Pharmacokinetic studies in man*” (3CC3a, 1987),
- “*Requirements for the non clinical safety evaluation of pharmaceuticals*” (ICH M3 (R2), revised 2009),
- “*Guideline on strategies to identify and mitigate risks for first-in-man human clinical trials with investigational medicinal products*” (EMA/CHMP/SWP/28367/07),

- “*Exploratory IND studies*” (FDA, 2006),
- “*Concept paper on the development of a CHMP guideline on the non-clinical requirements to support early phase I clinical trials with pharmaceutical compounds*” (EMA/CHMP/SWP/91850/2006),
- “*Position paper on non-clinical safety studies to support clinical trials with a single microdose*” (EMA/CHMP/SWP/2599/02/Rev1)
- and the most important one is: “*Guideline on clinical investigation of medicinal products indicated for the treatment of psoriasis*” (EMA/CHMP/EWP/2454/02 corr)

### 3.2 Materials & Methods used for the early clinical studies of interest

*Molecule A* is an oral drug registered for a non-dermatological disease. Its pharmacological properties were found to be potentially useful for the treatment of psoriasis. This *molecule A* was selected based on its more favourable *per os* benefit/risk ratio and safety profile compared to other drugs of the same therapeutic class. *Drug A* was then reformulated in order to allow topical administration in a new indication. It was therefore tested for the first time in human by topical route in two clinical studies in order to explore whether or not it was well tolerated, with the potential to be effective in the treatment of *Psoriasis Vulgaris*. The first study was performed in healthy volunteers and the second one in psoriatic patients.

Both clinical study designs were **Phase I, single centre, randomised, investigator blinded, intra-individual comparison, and repeated dose** with no direct therapeutic benefit for the subjects. Both clinical investigations were conducted according to the *Declaration of Helsinki* principles and the *Good Clinical Practice* (GCP) after the concerned *Regulatory authorities* and the *Ethics Committee* gave their approval. All subjects signed their informed consent forms before starting the trial.

### 3.2.1 21-Day tolerance Phase I Human pharmacology in healthy volunteers

This trial was a *21-Day tolerance* Phase I Human pharmacology study performed on healthy volunteers (HVs), entitled: '*Evaluation of the cumulative irritation potential of Drug A cream in healthy subjects*'.

The study objectives were:

- **Primary safety objectives:**
  - Evaluation of the cumulative irritation potential of *Drug A* cream at three concentrations (1%, 3% and 5%).
  - Evaluation of the systemic safety by adverse event reporting, physical examination, vital signs and laboratory safety tests follow-up.
- **Secondary objective (exploratory objective):**
  - Quantification of skin distribution of *Drug A* using two methods (tape-stripping and skin biopsies).

Twenty-seven healthy male volunteers were included in the study and received the study products on their back. The six tested products consisted of:

- Three different concentrations of *Drug A cream* at 1%, 3% and 5%
- The vehicle of the investigational product (as a negative control)
- Two commercially available treatments as positive comparators for the local tolerance assessment

Study products were applied on six delimited small zones (Z1 to Z6) of approximately 3cm<sup>2</sup> each on the back, under non-occlusive conditions, in order to answer to the primary objectives. Two additional areas (AZ) dedicated for the skin samplings (secondary objective) were located on the upper back. The randomisation of those two zones was performed in order to have parallel groups of nine subjects per concentration of *Drug A* (1%, 3% and 5%). Ten µL of test products were applied daily (five days a week) during the two first weeks and from Monday to Thursday during the third week (thus 14 applications in 18 days) (*Figure 26*).

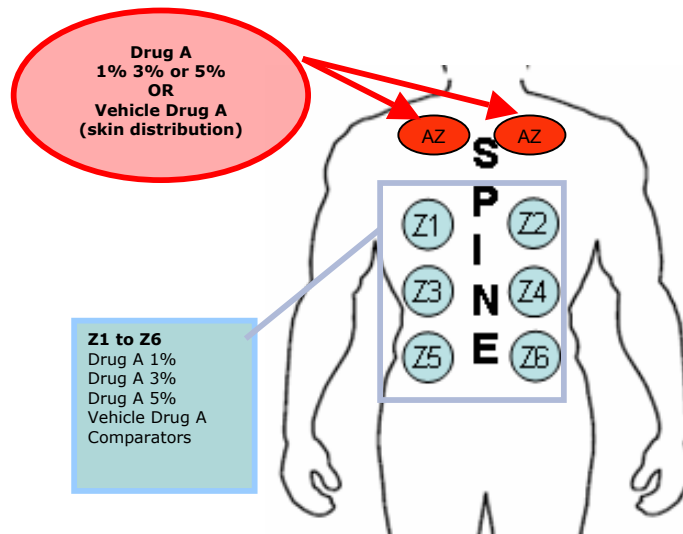


Figure n°26| **Study schematic.** (Adapted from Galderma documentation)

The main inclusion criteria were:

- Healthy Male Subject, 18 to 55 years old inclusive
- The subject is healthy as determined by the investigator based on a medical evaluation including medical history, physical examination, vital signs and laboratory tests done at the Screening visit
- The subject has understood and signed an Informed Consent Form as approved by the Ethics Committee, prior to screening evaluations.

The main non-inclusion criteria were:

- Subject with an underlying disease, surgical or medical condition, which, in the judgement of the investigator, could put the subject at risk or interfere with the study assessments.
- Abnormal or clinically significant laboratory tests
- Subject who did not respect the wash-out periods for medical treatments defined in the protocol
- Exclusion criteria related to the *Drug A* (known side effects, ingredient intolerance...)
- Exclusion criteria related to the biopsy (allergy to local anaesthetics, history of coagulation trouble or abnormal healing...)



### 3.2.2 PoC phase I Human pharmacology in psoriatic patients

This exploratory study was a Proof-of-Concept Phase I study indicated for the topical treatment of *Psoriasis Vulgaris*, entitled: '*Safety of Drug A cream in subjects with Psoriasis Vulgaris*'.

The study objectives were:

- **Primary objectives:**
  - Evaluation of the local tolerance of the study product using a 4-point scale tolerance score (twice a week) and systemic safety profile by adverse events reporting, physical examination, vital signs and laboratory safety tests, in order to determine the maximum tolerated concentration in the target population.
- **Secondary objectives:**
  - Clinical evaluation of the efficacy of *Drug A* at any concentration in comparison with the vehicle and calcipotriol: Total Sum Score<sup>14</sup> (TSS) + clearing score<sup>15</sup> performed twice a week.
- **Other objectives (or experimental objectives):**
  - Quantification of the skin distribution using two methods: tape stripping and skin biopsies performed at the end of the trial.

Clinical hypothesis concerning experimental objectives:

- As low systemic exposure was expected following topical application on a limited area, no blood samplings were taken for systemic PK measurements.
- One *in-vitro* liberation-penetration study had been conducted, using human skin. The results (*See section 4.3*) showed skin distribution of *Drug A* at 17%, 7% and 6% of the applied dose recovered in the skin after 16-hour topical application of the cream at 1, 3 and 5% respectively, suggesting a decrease of drug penetration as concentration increases.
- However, there were no clinical data available on the quantity of *Drug A* in the skin after topical applications. This is why it was decided to determine *Drug A*'s levels in psoriatic skin by performing tape-stripping and a 3-mm skin biopsy at

<sup>14</sup> **TSS:** The total sum Score is defined as the sum of Erythema (0-4), Scaling (0-4), and Thickness (0-4) scores.

<sup>15</sup> **Clearing score** defines the clearing of the psoriatic zone. It is divided into 3 scores (3-point scale): complete clearing, almost clear and unchanged

the end of the treatment period on patients who agreed to undergo skin biopsy and who were treated by *Drug A* 1% and 5%.

Twenty-two patients with stable chronic psoriasis plaque were included in the study. The six tested products included:

- Four different concentrations of *Drug A cream* at 1%, 2%, 3% and 5%
- The vehicle of the investigational product (as a negative control)
- A commercially available treatment as a positive comparator, calcipotriol 0.005% cream, which is a vitamin D derivative known to be effective in the treatment of psoriasis.

The study products were tested in a '*modified Dumas Scholtz test*' (Baadsgaard, O. & al., 1990) (the *Dumas Scholtz's test* is described in the *Literature Review*). The modification involves the application of treatments in a non-occlusive manner (by using a non-occlusive gauze between applications) in order to be closer to 'real life' conditions. The products were randomly allocated to six sites of 2-cm diameter on one or more plaques of similar severity (similar Total Sum Score, explained below) located on the legs, arms or trunk. Fifty  $\mu\text{L}$  ( $16\text{mg}/\text{cm}^2$ ) of study products were applied once a day on each application site of  $3\text{ cm}^2$ . The duration of the participation was a maximum of 3 weeks of screening period and 3 weeks of treatment (6 days/week, i.e. a total of 18 applications). A one-week follow-up period was added for subjects who accepted cutaneous PK samplings (*Figure 27*).

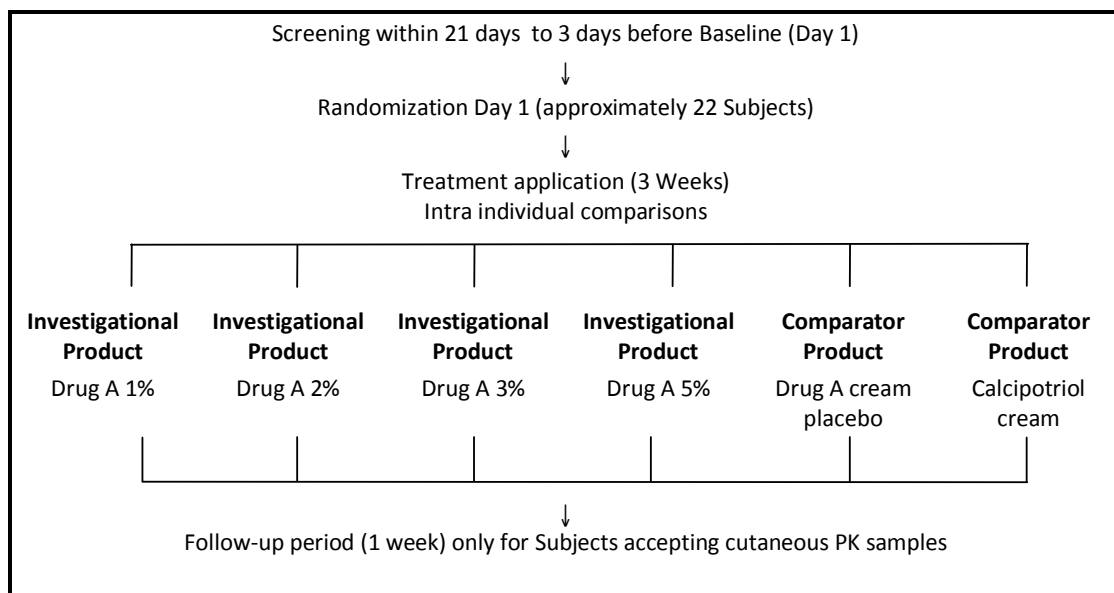


Figure n°27 | **Study schematic.** (Adapted from Galderma documentation)

The main inclusion criteria were:

- Male or female patients aged 18 to 70 years with stable chronic psoriasis plaque presenting:
  - Six target sites of 2-cm diameter on one or more psoriasis plaques on the legs, trunk or arms
  - With a Total Sum Score (TSS) between 4 and 9 inclusive on a 13 point scale (0-12)
  - With similar severity: TSS at Baseline +/- 1 grade.
  - Are at least 2 cm apart from each other
- Female of childbearing potential had to have an effective method of contraception for at least 4 weeks prior to screening visit and during the study.
- The subject had to agree to comply with the requirements of the protocol, to understand and sign an informed consent form at screening visit before any investigational procedure, +/- an additional form for those subjects who agreed to participate to the cutaneous sampling (tape-stripping and skin biopsy).

The main non-inclusion criteria were:

- Subject with an underlying disease which, in the investigator's opinion, would put the subject at risk or interfere with the study assessments
- Subject with a type of psoriasis other than *Psoriasis Vulgaris*
- Any medical history of hepatic or renal disease
- Exclusion criteria related to the *Drug A* (known side effects, ingredient intolerance...)
- Exclusion criteria related to the biopsy (allergy to local anaesthetics, history of coagulation trouble or abnormal healing...)
- Pregnancy
- Lastly, some wash-out periods were defined before the study for topical and systemic treatments of psoriasis
- Abnormal and clinically significant laboratory tests

### 3.2.3 Evaluation tools used for dermal measurements

The two chosen methods to determine skin distribution of *Drug A* in the different layers of the skin (Stratum corneum and Epidermis/Dermis) were tape stripping and skin punch biopsy. These two techniques are detailed in the *Literature review* (section 1.4.2.3.). At the final visit, 24-hours after the last application, the blinding was partially broken in order to determine the zone treated by *Drug A* 1%, 3% and 5% (in HVs study) or 1% and 5% (in psoriatic patients study) for the subjects who accepted such procedure.

#### Tape stripping

The tape stripping was performed on Day 19 (i.e. day after the last product application) for healthy volunteers in order to determine the *Drug A* accumulated concentration in the Stratum corneum after 14 applications. After removing the excess of formulation, tape stripping was done using a non-invasive adhesive device (Figure 28). Twenty strips were consecutively applied on one zone treated either by *Drug A* 1%, 3% or 5%. The strips (except the first one) were pooled two by two for the determination of *Drug A* concentration.

For psoriatic patients, the tape stripping was performed in the same condition as for healthy volunteers, but on Day 22 (i.e. day after the last product application), after 18 applications. Ten strips were consecutively applied on one zone treated by *Drug A* 1% or 5% instead of twenty strips in the usual procedure (on healthy skin). This was decided to limit the *Koebner Response*<sup>16</sup> on psoriasis plaques.

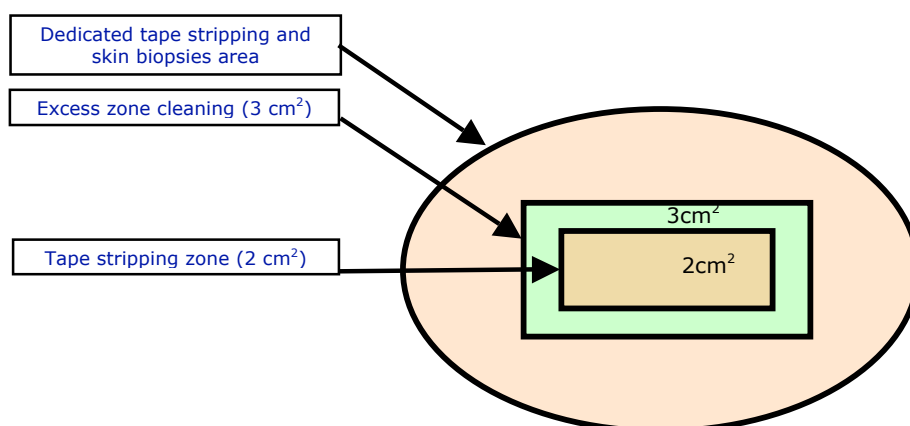


Figure n°28| **Tape stripping: Technical settings.** Source : Galderma documentation

<sup>16</sup> **The Koebner phenomenon** can be obtained by repeatedly applying and removing a piece of tape from the individual's skin (tape stripping), which affectively disrupt the skin barrier and leads to inflammation.

### **Skin biopsies**

On Day 19 for healthy volunteers and on Day 22 for psoriatic patients, a 3-mm punch skin biopsy was taken on the zone previously stripped, in order to determine *Drug A* concentration in the epidermis and dermis together. The procedure was done after disinfection and local anaesthesia of the biopsy area, using chlorhexidine and an intra-dermal injection with a Xylocaine-Adrenaline 1% solution. Each skin biopsy was then placed in a plastic tube and stored at -20°C until analysis. A follow-up visit was performed one week after to ensure the good healing of the biopsy.

The concentrations of *Drug A* were determined in strips and biopsies using a LC-MS/MS method. The limit of quantification was 1ng/mL.

### **3.3 Materials & Methods of the non-clinical study of interest**

Earlier in the development of *Drug A*, a liberation-penetration study was performed on excised human skin using a *Franz* type diffusion cell (the model and technique are described in the *Literature Review section 1.3.2.2*). Such studies allow the measurement of drug penetration into the skin after topical application.

The study title was: '*Comparison of the in-vitro liberation-penetration of Drug A formulated at 1%, 3% and 5% cream through Human skin*'.

#### Study objectives:

- Comparison of *in-vitro* percutaneous penetration of *Drug A* through human non-viable skin (excised human skin)
- Support of safety margin calculation

#### Study design:

The *in-vitro* liberation-penetration study (also called percutaneous absorption study) of *Drug A* was measured using the '*Finite Dose Technique*' (Franz, T.J., 1978). For instance, a single dose of the product was applied on ex-vivo full thickness human skin: six *Franz* type diffusion cells of 2 cm<sup>2</sup> (three donors, two samples per donor)

received 2mg/cm<sup>2</sup> of Drug A 1%, 3% or 5%. After 16-hours of exposure, the following compartments were analysed with LC/MS/MS (*cf Literature Review, section 1.3.2.2.*):

- Excess
- Stratum corneum
- Epidermis
- Dermis
- Receptor fluid

The limit of quantification was 1ng/ml.

### 3.4 Statistical analysis of the skin distribution results

The *Drug A* (Epidermis + Dermis) concentration was the only variable analysed by the statistics department. The results that will be presented in the next chapter correspond to experimental post-hoc analyses performed by the clinical pharmacokinetics department. The following variables were summarised using descriptive statistics (Mean, Standard Deviation, Min, Max, N) for *Drug A* 1%, 3% (except for psoriatic patients) and 5%:

- *Drug A* cumulated concentration in the SC, the (E+D) and in the Total Skin (SC+E+D)
- *Drug A* concentration profile to determine drug distribution into the SC

Standard Deviation (SD) will be used to construct confidence intervals for the means. The Coefficient of Variation (CV = SD/ Mean, expressed as percentage) is a normalised measure of dispersion of a probability distribution, which represents the knowledge of the assay variability. CV is a dimensionless number that allows comparison of variability estimates regardless of the magnitude of the means and the units of data sets.

## Chapter 4: RESULTS –

### 4.1 Summary of the skin distribution results in healthy volunteers

#### 4.1.1 Introduction

Twenty-seven healthy volunteers (one of whom dropout) participated in the *21-Day tolerance study* entitled ‘*Evaluation of the cumulative irritation potential of Drug A cream in healthy subjects*’. Whereas primary objectives were local and systemic safety evaluation, secondary objectives included the quantification of *Drug A* in the different layers of the skin on *Day 19* (24-hours after last applications) on one dedicated zone, treated either with *Drug A* 1%, 3% or 5% (8-9 subjects per group). Tape-stripping followed by a skin biopsy was performed on eight patients treated with *Drug A* 1%, nine with *Drug A* 3% and nine with *Drug A* 5%. All skin samples (strips and biopsies) were quantifiable (with a limit of quantification of 1ng/mL). Tape-stripping and skin biopsy methods measure the drug concentration in the Stratum Corneum and in the (Epidermis + Dermis) respectively. Those methods are detailed in the *Literature Review, Section 1.4.2.3*. The protocol followed during the clinical trial for skin distribution measurements is described in the *Materials & Methods, Section 3.2.3*.

#### 4.1.2 Key results

Less than 10% of *Drug A* applied dose penetrated in the Total Skin (i.e. Stratum Corneum (SC) + Epidermis (E) + Dermis (D)), and approximately 1% of the applied dose penetrated in the deep skin layers (i.e. E+D), in which the site of action is located. Concentrations (in  $\mu\text{g}/\text{cm}^2$ ) in the (E+D) increased with *Drug A* dosage increase and were statistically compatible with dose-proportionality. In contrast, concentrations in the SC were not proportional to *Drug A* dosage.

The following *Table X* summarises skin distribution results of *Drug A* (Mean concentration  $\pm$  SD,  $\mu\text{g}/\text{cm}^2$  and percentage (%) of the applied dose that penetrates in the different layers), according to *Drug A dosage*. Detailed results are found in *Appendix A*. Graphs will then illustrate those results (*Figure 29 & 30*).

### 4.1.3 Detailed results

<b>Drug A</b>	<b>1%</b>	<b>3%</b>	<b>5%</b>
<b>Applied dose (<math>\mu\text{g}/\text{cm}^2</math>)</b>	32	96	160
<b>N</b>	8	9	9
<b>Stratum Corneum</b>			
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	1.32 $\pm$ 0.93	7.29 $\pm$ 7.11	3.34 $\pm$ 4.62
CV%	70%	98%	138%
% of applied dose	4.4%	7.6%	2.1%
<b>Epidermis + Dermis</b>			
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	0.24 $\pm$ 0.17	0.64 $\pm$ 0.60	0.83 $\pm$ 0.56
CV%	69%	94%	68%
% of applied dose	0.8%	0.7%	0.5%
<b>Total skin: SC+E+D</b>			
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	1.57 $\pm$ 0.96	7.93 $\pm$ 7.21	4.16 $\pm$ 4.79
% of applied dose	4.9%	8.3%	2.6%

Table X | **Drug A** skin distribution in the SC, the (E + D) and in the total skin, according to **Drug A** dosage.  
CV: Coefficient of Variation; SD: Standard Deviation; N: Number of samples. Source: Galderma documentation

#### 4.1.3.1 Drug A cumulated concentration in the SC, the (E+D) and in the Total Skin (SC+E+D):

The following graph shows *Drug A* skin distribution on Day 19, after 14 applications of the investigational product (once a day application, 5 days a week). *Drug A* concentration in the Stratum Corneum and in the deeper layers (Epidermis + Dermis) was determined by tape-stripping followed by skin biopsy procedures.

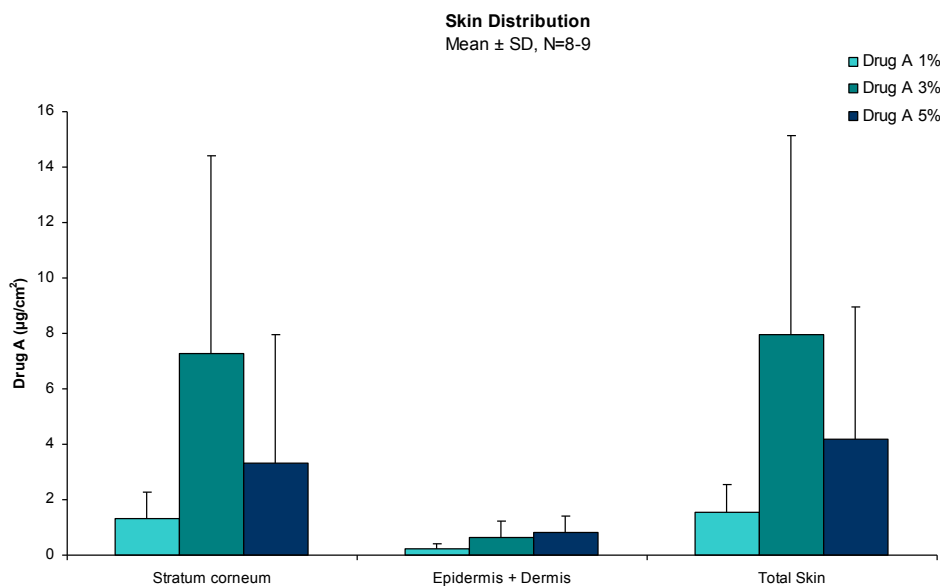


Figure n°29 | **Drug A** cumulated concentration in the SC, the (E+D) and in the total skin (SC+E+D), according to **Drug A** dosage, at Day 19. Adapted from Galderma documentation.



Several observations can be done on these results:

- Approximately 3 to 8 % of the applied dose was recovered in the Total Skin after repeated daily application (14 applications in total).
- Inter-individual variability (CV) was ranging from 68 to 138%. It was lower in the (E+D) than in the SC.
- Skin distribution\* (*see appendices E & F*):
  - 73- 84% of the penetrated dose (Total skin) was recovered in the SC.
  - The concentration was approximately 5 to 20-fold higher in the SC than in the (E+D).

\* the above percentages are calculated based on individual data and not based on mean data for the groups.

- Dose proportionality?
  - Dose proportionality was statistically demonstrated (data not shown) in the (E+D), which is the compartment of interest (*Drug A's* target site).
  - No statistical test was performed on SC results, but it seems that there was no dose proportionality between the dose applied on the skin surface and the concentration found in the SC.

#### 4.1.3.2 *Drug A* concentration profile to determine drug distribution in the SC

After the tape-stripping procedure, strips were pooled two by two, except the most superficial one (representing the excess of formulation) which was discarded in the analysis. The overall mean of *Drug A* concentration in the strips decreased significantly between strips #2-3 and strips #18-19-20 (*Figure 30 + detailed values in Appendix A*).

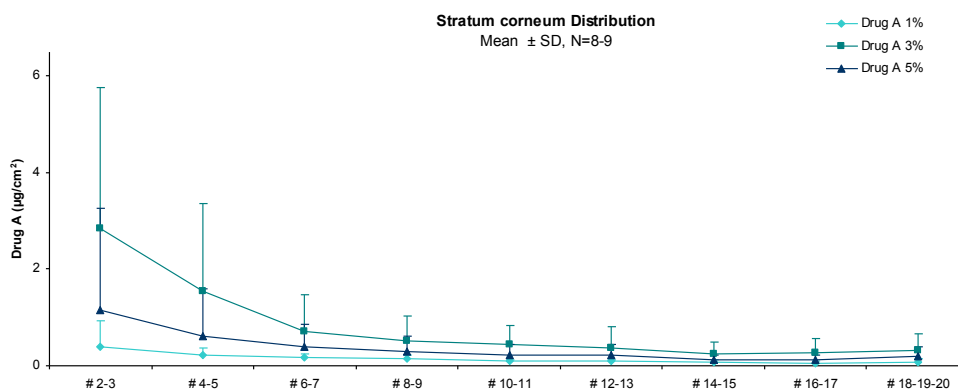


Figure n°30 | *Drug A* Stratum corneum distribution, according to *Drug A* dosage, at Day 19.  
Adapted from Galderma documentation.

As expected, concentrations were higher in the outer layers than in the deeper layers of the SC. Interestingly, *Drug A* concentrations were higher with *Drug A* 3% dosage than with the 5%; certainly due to high inter-individual and procedure variability.

## 4.2 Summary of the skin distribution results in psoriatic patients

### 4.2.1 Introduction

Twenty-two psoriatic patients participated in the three weeks *Proof-of-Concept* study entitled “*Safety of Drug A cream in subjects with Psoriasis*”. This clinical trial evaluated the safety, efficacy and, for a subset of patients, the skin distribution of the investigational compound *Drug A* on Day 22 (24-hours after last applications). Tape stripping followed by a skin biopsy was performed on three patients treated with *Drug A* 1% and on four patients treated with *Drug A* 5%. All skin samples (strips and biopsies) were quantifiable (with a limit of quantification of 1ng/mL). Tape-stripping and skin biopsy methods are detailed in the *Literature Review, Section 1.4.2.3*. The protocol followed during the clinical trial for skin distribution measurements is described in the *Materials & Methods, Section 3.2.3*.

### 4.2.2 Key results

In the total skin (*i.e.* Stratum Corneum (SC) + Epidermis (E) + Dermis (D)): approximately 7% of the applied dose penetrated in *Drug A* 1% samples (Strips + biopsies) and 16% in the *Drug A* 5% samples. Approximately 0.35% of *Drug A* 1% & 5% applied dose penetrated in the skin deeper layers (*i.e.* E + D in the skin biopsy). The mean value of the penetrated concentration in the (E + D) was five times higher with *Drug A* 5% than with *Drug A* 1%: respectively  $2.83 \pm 3.65 \mu\text{g}/\text{cm}^2$  and  $0.60 \pm 0.80 \mu\text{g}/\text{cm}^2$ . Despite the high variability observed in this study, the results showed a dose proportionality of *Drug A* penetration in the deeper layers (which are the target sites), (see Figure 32a).

The following Table XI summarises skin distribution results of *Drug A* (Mean concentration  $\pm$  SD,  $\mu\text{g}/\text{cm}^2$  and percentage (%) of the applied dose that penetrates in the different layers), according to *Drug A* dosage. Detailed results are found in Appendices B, C & D. Graphs will then illustrate those results (Figure 31-34).

### 4.2.3 Detailed results

<b>Drug A</b>	<b>1%</b>	<b>5%</b>
<b>Applied dose (<math>\mu\text{g}/\text{cm}^2</math>)</b>	166	833
<b>N</b>	3	4
<b>Stratum Corneum</b>		
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	11,13 $\pm$ 9,32	131,10 $\pm$ 89,82
CV%	84%	69%
% of applied dose	<b>6.70%</b>	<b>15.74%</b>
<b>Epidermis + Dermis</b>		
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	0,60 $\pm$ 0,80	2,83 $\pm$ 3,65
CV%	134%	129%
% of applied dose	<b>0,36%</b>	<b>0,34%</b>
<b>Total skin: SC+E+D</b>		
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	11,72 $\pm$ 10,12	133,93 $\pm$ 93,15
% of applied dose	<b>7.06%</b>	<b>16.08%</b>

Table XI | **Drug A** skin distribution in the SC, the (E + D) and in the total skin, according to **Drug A** dosage. CV: Coefficient of Variation; SD: Standard Deviation; N: Number of samples. Adapted from Galderma documentation.

#### 4.2.3.1 Drug A cumulated concentration in the SC, the (E+D) and the Total Skin (SC+E+D):

The following graphs show the *Drug A* skin distribution on Day 22, after 18 applications of the investigational product (once a day application, 6 days a week). *Drug A* concentration in the Stratum corneum and in the deeper layers was determined by tape-stripping followed by skin biopsy procedures.

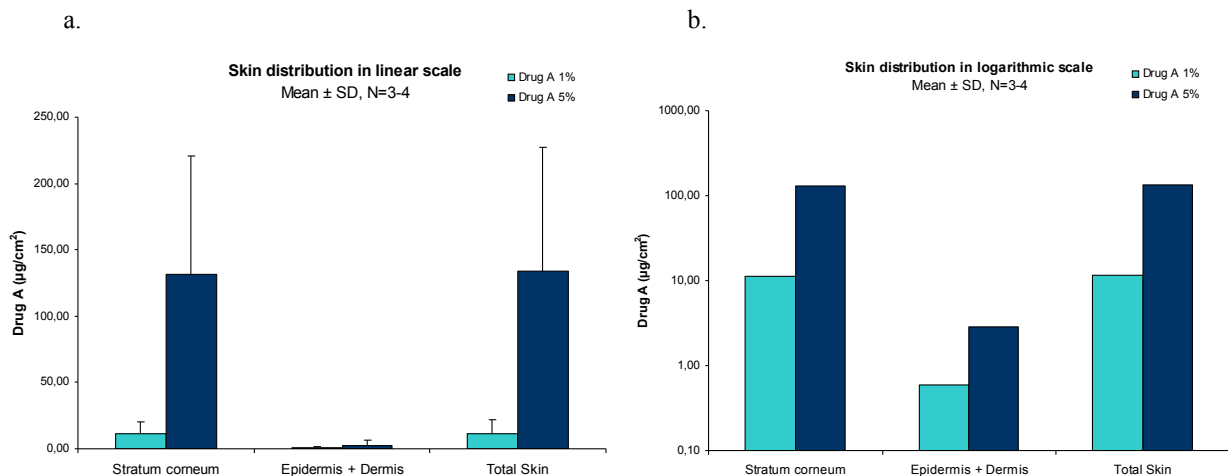


Figure n°31 | **Drug A** accumulated concentration in the SC, (E+D) and the total skin, according to Drug dosage, at Day 22. a. linear scale; b. logarithmic scale. Adapted from Galderma documentation.

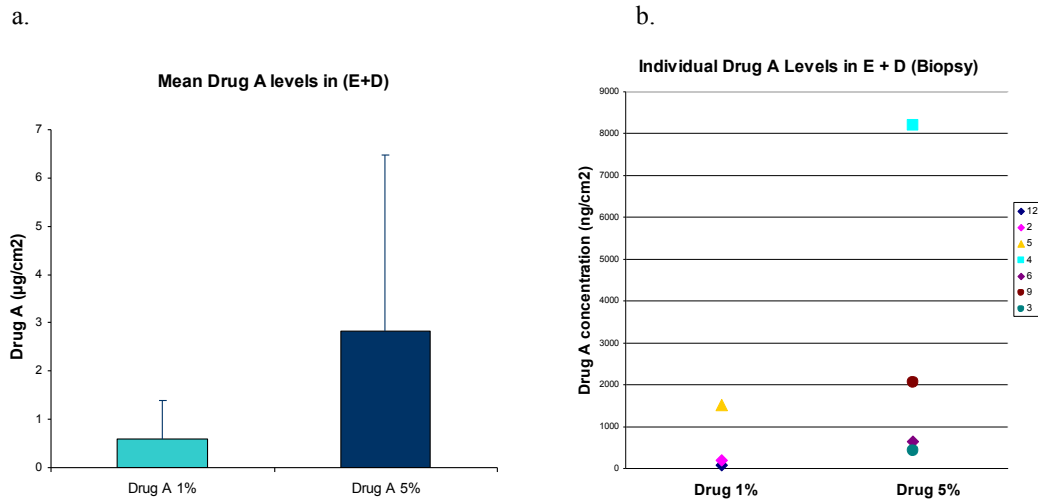


Figure n°32 | (E+D) *Drug A* concentration according to *Drug dosage*, at Day 22. a. Mean *Drug A* concentration + error bars; b. Great disparities between individual *Drug A* levels in the (E+D). Adapted from Galderma documentation.

Several observations can be done on these results:

- 7 to 16% of the applied dose was recovered in the Total Skin after repeated daily applications (18 applications in total).
- Inter-individual variability ranged from 69 to 134%
- Skin distribution\* (*see appendices E & F*):
  - Higher accumulation of *Drug A* in the SC than in (E+D) with more than 95% of the penetrated dose in the SC.
  - It was particularly marked with the 5% dosage: *Drug A* level was 115-fold higher in the SC than in the (E+D)

\* the above percentages are calculated based on individual data and not based on mean data for the groups.

- The *Drug A* cumulated concentration and the percentage of the applied dose that penetrated in the total skin (SC + E+ D) was almost identical to the SC's ones because very little (~0.35%) went to the deeper layers (independently of *Drug A* dosage), that is:
  - *Drug A* 1% samples:  $11.72 \pm 10.12 \mu\text{g}/\text{cm}^2$ , corresponding to approximately 7% (6.70% (SC) + 0.36% (E + D)) of the applied dose.
  - *Drug A* 5% samples:  $133.93 \pm 93.15 \mu\text{g}/\text{cm}^2$ , corresponding to approximately 16% (15.74% (SC) + 0.34% (E + D)) of the applied dose
- Dose proportionality?
  - Despite high variability between individual samples (*Figure 32b* + error bars *Figure 32a* + *Appendices C & D* for individual values) dose

proportionality was achieved in the Epidermis and Dermis together. The mean concentration was five times higher with *Drug A* 5% than with *Drug A* 1%:  $2.83 \pm 6.65 \mu\text{g}/\text{cm}^2$  and  $0.60 \pm 0.80 \mu\text{g}/\text{cm}^2$  respectively (Figure 32a).

- No statistical test was performed on SC results, but it seems that there was no dose proportionality in the Stratum Corneum: the mean concentration of *Drug A* 5% ( $131.10 \pm 89.82 \mu\text{g}/\text{cm}^2$ ) was approximately twelve times higher than the mean concentration of *Drug A* 1% ( $11.13 \pm 9.32 \mu\text{g}/\text{cm}^2$ ).

#### 4.1.3.2 *Drug A* concentration profile to determine drug distribution in the SC

The strips were pooled two by two, except the most superficial one (representing the excess of formulation) which was discarded in the analysis. The overall mean and the individual concentration of *Drug A* in the strips decreased significantly between strips #2-3 and strips #8-9 (Figure 33, Figure 34a & 34b + detailed values in Appendix B). As expected, concentrations were higher in the outer layers than in the deeper layers of the SC. Although there was notable inter-individual variability, the trend was similar between patients (Figure 34a & 34b + Appendices C & D for individual values). Moreover, individual *Drug A* 5% SC distributions results were higher than the 1%'s ones (the *y* axis scale is different between Figure 34a & 34b), showing a high accumulation of the *Drug A* 5% in the SC. There was one outlier (patient n°4) with an abnormal mean concentration for the strips #4-5. This outlier value was ignored in the analysis (Figure 34b).

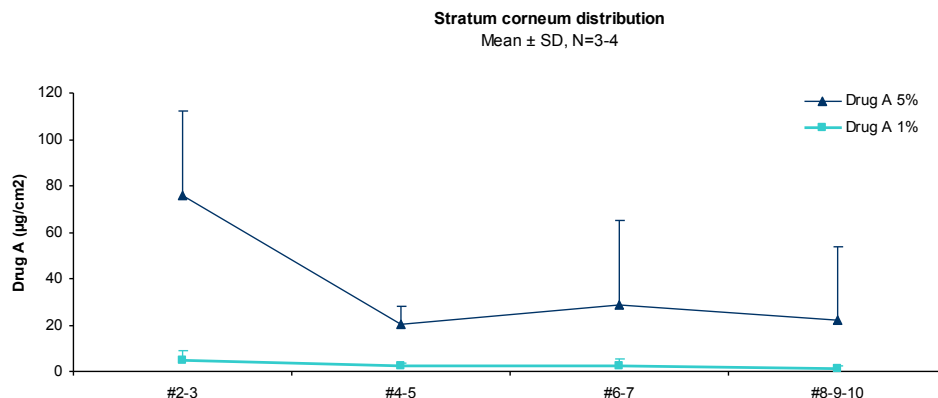
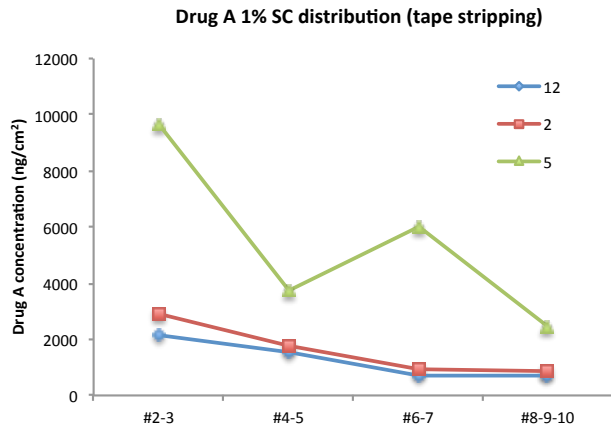


Figure n°33 | ***Drug A* Stratum corneum distribution, according to *Drug A* dosage, at Day 22.**  
Adapted from Galderma documentation.

a.



b.

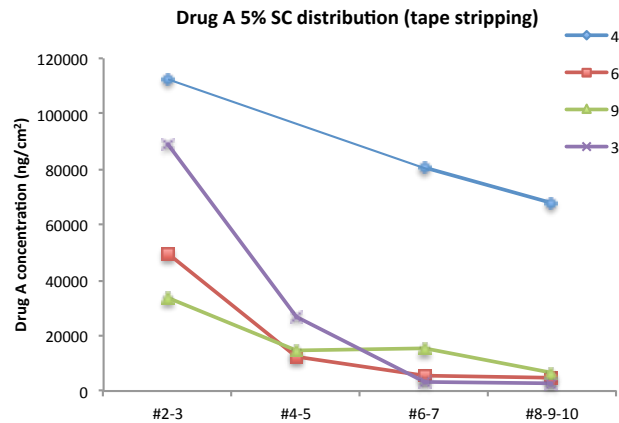


Figure n°34 | **a.** Individual *Drug A* 1% SC distribution (patients n° 2, 5 and 12). **b.** Individual *Drug A* 5% SC distribution (patients n° 3, 4, 6 and 9). Adapted from Galderma documentation.

### 4.3 Summary results of *in-vitro* liberation penetration study

Excised human skin samples from three donors were used in a liberation-penetration study entitled: “*Comparison of the in-vitro liberation-penetration of Drug A 1%, 3% and 5% through excised human skin*”. This study compared *in-vitro* liberation penetration of *Drug A* at 1%, 3% and 5% through human non-viable skin and its principal objective was to support safety margin calculation.

The following *Table XII* summarises skin distribution results of *Drug A* (Mean concentration  $\pm$  SD,  $\mu\text{g}/\text{cm}^2$  and percentage (%) of the applied dose that penetrates in the different layers), according to *Drug A* dosage.

<i>Drug A</i>	1%	3%	5%
Applied dose ( $\mu\text{g}/\text{cm}^2$ )	17.93	60.15	97,9
N	6	6	6
<b>Stratum Corneum</b>			
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	2.72 $\pm$ 1.17	3.55 $\pm$ 2.15	4.49 $\pm$ 2.57
CV%	43.3%	60.7%	57.4%
% of applied dose	15.3%	5.9%	4.8%
<b>Epidermis + Dermis</b>			
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	0.33 $\pm$ 0.18	0.62 $\pm$ 0.55	1.23 $\pm$ 0,62
CV%	55.1%	89.7%	50.7%
% of applied dose	1.9%	1.0%	1.3%
<b>Total skin: SC+E+D</b>			
Mean $\pm$ SD ( $\mu\text{g}/\text{cm}^2$ )	3.05 $\pm$ 1.18	4.17 $\pm$ 2.38	5.72 $\pm$ 2.84
% of applied dose	17.2%	6.9%	6.1%

Table XII | *Drug A* skin distribution in the SC, (E + D) and the total skin, according to *Drug A* dosage. CV: Coefficient of Variation; SD: Standard Deviation; N: Number of samples. Adapted from Galderma documentation.

The following graph shows *Drug A* skin distribution 16h-hours after a single application of the investigational product. *Drug A* concentration in the SC was determined after tape stripping. The level in the Epidermis and Dermis was measured separately after dissociating these two layers with a scalpel.

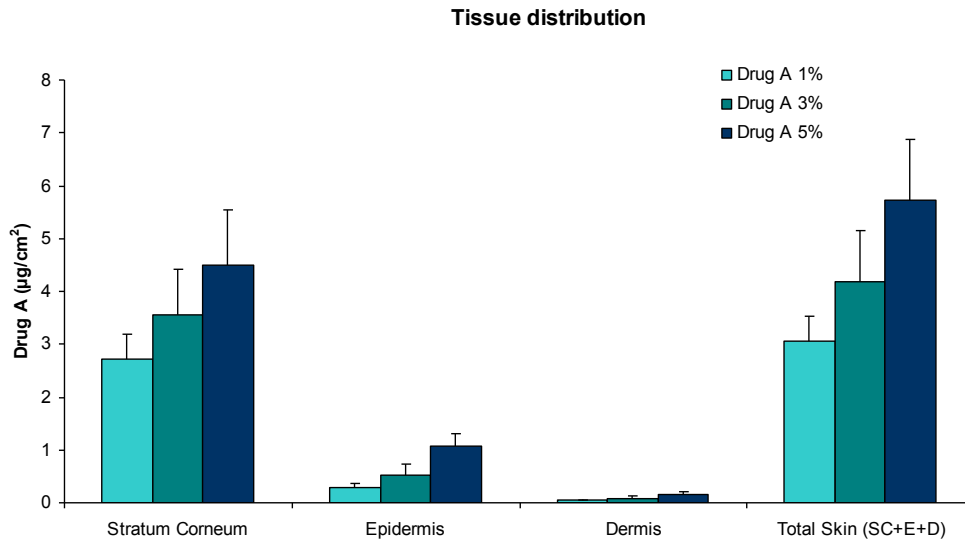


Figure n°35 | *Drug A* accumulated concentration in the Stratum Corneum, the Epidermis, the Dermis and in the Total Skin, according to *Drug* dosage, at Day 22. *a.* linear scale; *b.* logarithmic scale. Adapted from Galderma documentation.

Several observations can be done on these results:

- 6 to 17% of the applied dose was recovered in the Total Skin after a single application (16-hours of incubation).
- CV were ranging from 43 to 89%
- Skin distribution (*see appendices E & F*):
  - High exposition in the SC: 78-89% of the penetrated dose (Total skin) was recovered in the SC.
  - Lower penetration in the Epidermis + Dermis: *Drug A* levels were approximately 3 to 8-fold higher in the SC than in the (E+D).
- Dose proportionality:
  - No statistical test was performed on SC results, but it seems that there was no dose proportionality between the dose applied on the skin surface and the concentration found in the SC.

- Although not proportionally, total penetration (including SC) of *Drug A* increased with *Drug A* dosage.
- Data were statistically compatible with dose proportionality in the Epidermis + Dermis layers (data not shown).



## Chapter 5: DISCUSSION –

### 5.1 Introduction

These exploratory findings are of great interest in early development. First, they contributed to the validation of refined dermal evaluation tools and secondarily, they provided information on penetration profile of *Drug A in-vitro*, in healthy volunteers and in the target patients. This precious skin distribution information supported early decision-making. The following tables summarise skin distribution results in the three studied models (*Table XIII*).

a.

Skin concentrations	<i>In-vitro</i> data (Liberation-penetration study)			Clinical data on healthy skin			Clinical data on psoriatic skin	
	<i>Drug A</i> 1%	<i>Drug A</i> 3%	<i>Drug A</i> 5%	<i>Drug A</i> 1%	<i>Drug A</i> 3%	<i>Drug A</i> 5%	<i>Drug A</i> 1%	<i>Drug A</i> 5%
Quantity applied ( $\mu\text{g}$ )	36	120	196	32	96	160	166	833
SC ( $\mu\text{g}/\text{cm}^2$ )	2.72	3.55	4.49	1.32	7.29	3.34	11.13	131.10
E+D ( $\mu\text{g}/\text{cm}^2$ )	0.33	0.62	1.23	0.24	0.64	0.83	0.60	2.83
SC+E+D ( $\mu\text{g}/\text{cm}^2$ )	3.05	4.17	5.72	1.57	7.93	4.16	11.72	133.93

b.

% of the applied <i>Drug A</i>	<i>In-vitro</i> data (Liberation-penetration study)			Clinical data on healthy skin			Clinical data on psoriatic skin	
	<i>Drug A</i> 1%	<i>Drug A</i> 3%	<i>Drug A</i> 5%	<i>Drug A</i> 1%	<i>Drug A</i> 3%	<i>Drug A</i> 5%	<i>Drug A</i> 1%	<i>Drug A</i> 5%
SC	15.3%	5.9%	4.8%	4.4%	7.6%	2.1%	6.7%	15.4%
E+D	1.9%	1.0%	1.3%	0.8%	0.7%	0.5%	0.36%	0.34%
SC+E+D	17.2%	6.9%	6.1%	4.9%	8.3%	2.6%	7.1%	16.1%

Table XIII| a. Mean skin concentration in tape-strips, biopsies and total skin of three different models: ex-vivo human skin, healthy skin and pathological skin. b. Percentage of the applied dose that penetrates in the different layers of the skin for each model. Source: Galderma documentation.

The results showed that a high proportion of penetrated dose of *Drug A* was found in the SC rather than the deeper layers, as shown in the table after applications of *Drug A* on psoriatic skin:

- For *Drug A* 1%:  $11.13 \pm 9.32 \mu\text{g}/\text{cm}^2$  (7%) in the SC compared to  $0.6 \pm 0.8 \mu\text{g}/\text{cm}^2$  (0.36%) in the (E + D), and
- For *Drug A* 5%:  $131.10 \pm 89.82 \mu\text{g}/\text{cm}^2$  (16%) in the SC compared to  $2.83 \pm 3.65 \mu\text{g}/\text{cm}^2$  (0.34%) in the (E + D)

Such results are relevant since the SC barrier is the outer layer of the skin and represents the principal resistance to the penetration of topical dermatological agents. For instance, only a small quantity of drug product can cross this protective rate-controlling membrane to the deeper skin layers.

A cross analysis was carried out in order to compare the results above-presented. It will be structured as followed:

1. Clinical study on healthy skin (healthy volunteers) *versus* Clinical study on psoriatic skin (patients)
2. Overall cross analysis, comparing liberation-penetration *in-vitro* study on excised healthy human skin *versus* Clinical studies performed on healthy volunteers and psoriatic patients

As these studies were not specifically designed for this cross analysis, a number of differences in the designs remain, limiting proper comparison between studies. For instance, the major differences are the quantity of *Drug A* applied and the number of skin samples. The table below summarises the main differences between the three studies of interest (*Table XIV*). All these differences may impact the relevance of the results, except the number of tape-strips used since 90% of the concentration is usually found in the first ten tape-strips (*see Literature Review section 1.4.2.3*). Also, there is no possible comparison with psoriatic patients for the *Drug A* 3% samples since no samples were taken for this dosage (recruitment reasons).

<i>Design Comparison</i>	<b><i>In -vitro</i> Liberation-penetration study</b>	<b>Clinical trial in healthy volunteers</b>	<b>Clinical trial in psoriatic patients</b>
<b>Type of Skin</b>	<i>Ex-vivo</i> human skin (non-viable, no vasculature) Males/ Females	Healthy human skin (viable) Males	Psoriatic human skin (viable) Males/ Females
<b>Frequency of application</b>	Single application (16h of incubation)	Once a day application 5 days a week (14 applications in total)	Once a day application 6 days a week (18 applications in total)
<b>Quantity of cream to be applied</b>	2mg/cm <sup>2</sup> (4μL)	3.2mg/cm <sup>2</sup> (10μL)	16mg/cm <sup>2</sup> (50μL)
<b>Drug A applied dose (μg/cm<sup>2</sup>)</b>	97μg/cm <sup>2</sup> (for the 5%)	160μg/cm <sup>2</sup> (for the 5%)	833μg/cm <sup>2</sup> (for the 5%)
<b>Samples' dosage</b>	<i>Drug A</i> 1%, 3%, 5%	<i>Drug A</i> 1%, 3%, 5%	<i>Drug A</i> 1%, 5%
<b>Tape stripping procedure</b>	20 consecutive tape-strips After 16hours	20 consecutive tape-strips Day 19	10 consecutive tape-strips (to limit skin irritation) Day 22
<b>Number of samples (N)</b>	6 (2 cells per donor)	27 (9 samples per dosage)	7 (3 & 4 samples for <i>Drug A</i> 1% & 5% respectively)
<b>Location of the biopsies</b>	4 Mammary & 2 abdominal	Upper back	Legs, trunk or arms

Table XIV | Main differences in the design of the three studies. Adapted from Galderma documentation.

## 5.2 Clinical skin distribution data cross analysis: normal skin (healthy volunteers) versus psoriatic skin (patients)

### 5.2.1 Data comparison

The following graphs compare the percentage of the applied dose that penetrates in the SC and in the (E+D) of normal skin *versus* psoriatic skin, depending on *Drug A* dosage (*Drug A* 1% and 5%). Those results must be carefully evaluated knowing that the absolute quantity applied at each application was 5-times higher on psoriatic skin than normal skin: 50 μL *versus* 10 μL (see table XIV). Thanks to clinical experience, it is actually known that such quantity is well absorbed on psoriatic skin. If the same quantities were applied on HVs, too much excess would remain unabsorbed. Thus, we should compare percentages of the applied dose that penetrates rather than mean concentrations.

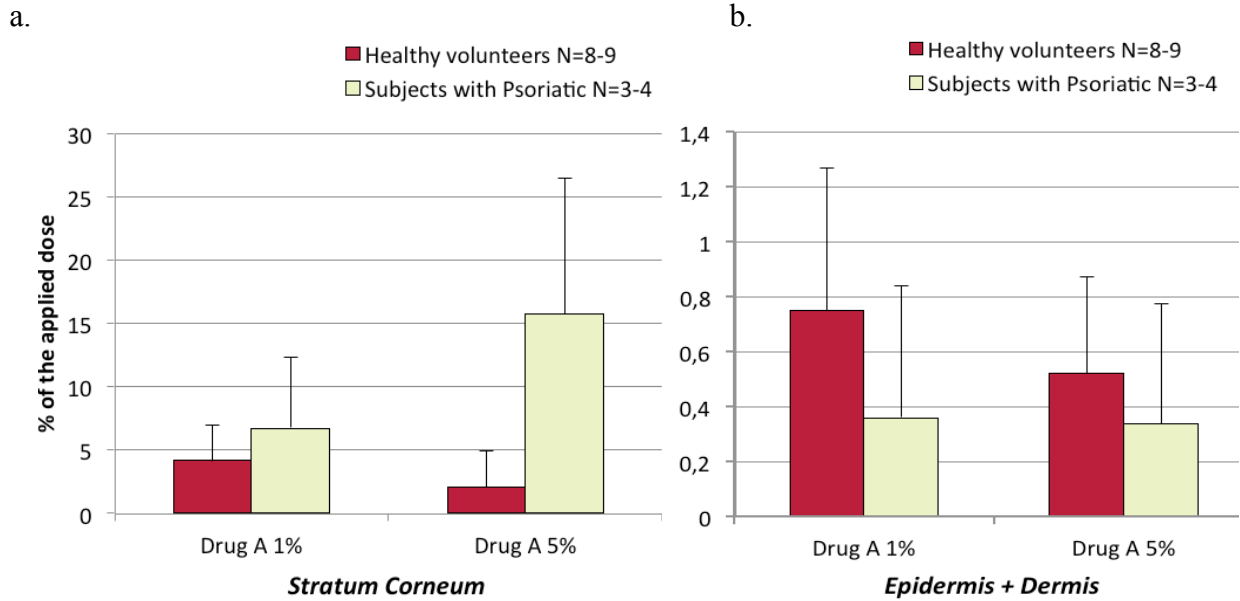


Figure n°36 | Comparison of skin penetration (in terms of % of the applied dose) in HVs versus subjects with psoriasis. **a.** in the SC. **b.** in the (E+D). Adapted from Galderma documentation.

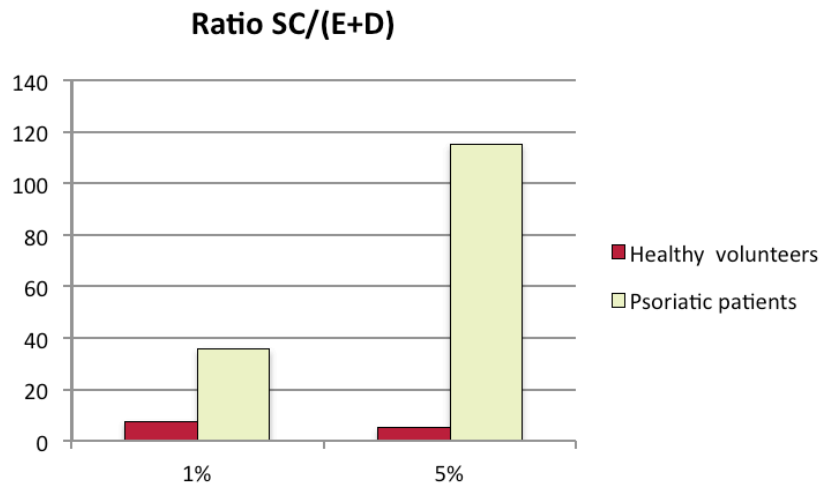


Figure n°37 | Ratio between Stratum Corneum and deeper layers *Drug A* concentration in HVs and subjects with psoriasis, depending on *Drug A* dosage. Adapted from Galderma documentation.

#### Normal skin versus psoriatic skin:

- Difference in skin distribution (*Figure 36a. & 37 + Appendix E for values*):
- *Drug A* concentration was much higher in the SC than in the (E+D) for both healthy volunteers and in psoriatic patients. This is explained by the SC barrier that limits penetration of xenobiotics through the deeper layers of the skin.

- However, the difference was more marked in psoriatic patients. For instance, in psoriatic patients, the concentration ratio (SC/E+D) was approximately of 35 to 115, meaning that *Drug A* concentration was approximately 35 to 115 times higher in the SC than in the (E+D), in opposition to healthy volunteers for whom the concentration ratio (SC/E+D) was approximately 5 to 7. Psoriasis' skin characteristics and/or the dose of *Drug A* applied may be the cause of such differences
- The SC *Drug A* levels in HVs skin were not representative of SC levels in psoriatic skin:
  - At *Drug A* 1%, the percentage of the dose applied that penetrated in the SC was relatively close in both models: 7% in psoriatic patients and 4.4% in HVs.
  - At *Drug A* 5%, the difference was much bigger with 16% of the applied dose that penetrated to the SC in psoriatic patients versus 2.1% in healthy volunteers.

The thicker SC as well as the type of cells found in psoriatic skin (*see next section 5.2.2*) seems to contribute to the accumulation of *Drug A* in the SC and may be conferred a reservoir effect that would explain the results. Moreover, the quantity applied on psoriatic skin was 5-times higher than the quantity applied on normal skin (50µL *versus* 10µL), which may saturate the SC.

- Limited impact of skin pathology on the E+D levels
  - Despite important error bars, the mean percentage of the applied dose that penetrated to the (E+D) stood between 0.35 and 0.75% for both models
  - The percentage was higher for healthy volunteers meaning that *Drug A* penetrates more easily in normal skin than in psoriatic skin.

Besides, one's should note that although the percentage of the applied dose that penetrates to the site of action is very low, the actual quantity that reaches the target is not that low since topical drugs are directly applied on the target organ.

### 5.2.2 Does psoriatic skin (i.e. pathological skin) impacts *Drug A* penetration? Could healthy volunteers be pharmacological models for psoriatic patients?

Psoriatic skin definitely impacts the penetration of a drug product compared to normal skin. Indeed, many different histological characteristics between psoriatic skin and normal skin can be noted (*Figure 38*).

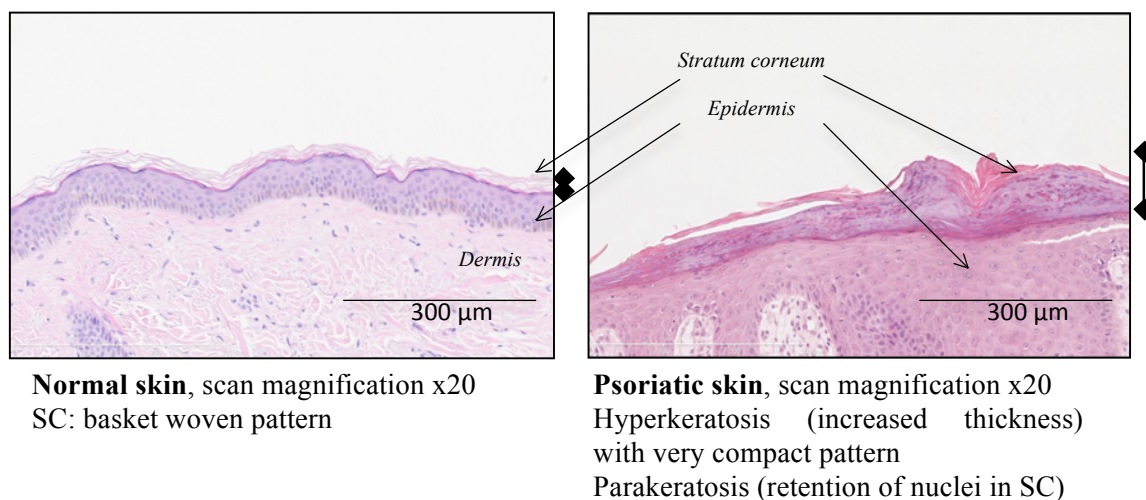


Figure n°38 | **Histology of a normal skin versus psoriatic skin.** Adapted from Galderma documentation.

Although the normal skin SC is characterised with loose dead cells (basket woven pattern), SC of psoriatic skin is very compact, meaning that the dead cells' junctions are very tight and make drug penetration difficult. Also the SC is thicker in psoriatic skin than normal skin (see double arrow *Figure 38*). The other modified histological structures of psoriatic skin are hyperkeratosis leading to epidermal acanthosis (increased thickness of the epidermis, see *Figure 38*), retention of nuclei in keratinocytes that reaches the SC (para-keratosis), leucocytes infiltrates and blood vessels dilation as well.

The above-mentioned characteristics found in psoriatic skin may explain most of the differences observed between healthy volunteers and psoriatic patients' penetration profile. The results suggested that *Drug A* penetration was lowered in

psoriatic skin, certainly because of the critical role of the SC as a reservoir (thickness of the SC) and as a barrier (compact pattern).

SC *Drug A* concentrations were not proportional to *Drug A* dosage probably due to high variability (CV), and potentially due to SC saturation and reservoir effect. Moreover, quantities applied in such designs (small zones of 3.14 cm<sup>2</sup>) are higher than in normal use. Accordingly to the results, this tendency was amplified in psoriasis patients, first by the large applied quantity of *Drug A*, second by the pathology. However, the (E+D) *Drug A* level was less impacted by the disease, dose proportionality was statistically found in both models. The percentage of the applied dose that penetrated in the (E+D) was nevertheless a higher (almost 2-times higher) in normal skin than in psoriatic skin.

Such observations are interesting since *Drug A* is intended to have its target site in the epidermis and the dermis. According to these results we can say that the SC concentration is not predictive of the deeper layers concentration. Indeed, the tape stripping results are informative but not essential since the target site is not in the SC. The SC potentially accumulates the drug product and releases it slowly and progressively to the deeper layers. Although the % of applied dose that penetrates in the (E + D) layers in healthy volunteers and psoriatic patient are in the same range between 0.34% and 0.75% (*Figure 36b*), healthy human volunteers do not seem to be a relevant model to mimic the *Drug A* skin penetration profile of psoriatic patients. Even among the psoriatic population, inter-individual variability is consequent and influences penetration profile of topical dermatological agents.

### **5.2.3 Limits in the interpretation.**

The interpretation of these results is however limited by their coefficients of variation (CV, expressed in %). The variability can be explained first by the limited number of skin samples (especially in the psoriatic patients study), by inter & intra-individual variations and finally by variations related to the procedure and the bioanalysis. These are discussed in the following paragraphs.

Variations related to the number of skin samples: The number of samples was a bit low in the psoriasis study to obtain significant results (three skin biopsies for the *Drug A* 1% and four for the *Drug A* 5%). These exploratory results are interesting as a preliminary approach as they offer a “snapshot” of the drug dermal distribution in the different skin layers and may support the *Go/NoGo decision*.

Inter & intra-individual variations: Every individual has intrinsic skin particularities that can influence penetration of an applied topical agent (e.g. skin histology, thickness of the skin etc...). Even for a same individual, skin characteristics change depending on the body part. In psoriatic patients, skin characteristics are different depending on the severity of the psoriatic plaques. The only thing we can control in psoriatic patients is the choice of the plaque that undergoes the biopsy. For example, the selection of plaques with ‘similar’ severity (*i.e.* same range of TSS, see *Materials & Methods chapter*) and an equivalent body part location among patients could be specified in the inclusion criteria. Meanwhile, information on the location of the biopsy should be collected.

Variations related to the procedure: As introduced in the *Literature Review chapter, section 1.4.2.3*, the tape stripping method may be associated with lack of standardisation. For instance, the centre where this clinical trial was conducted did not have any previous expertise in the tape stripping technique, leading to a potential bias linked to the manipulator for example (especially if it was not the same nurse who performed all the strippings). A similar observation can be made for the biopsy technique. Its procedure can certainly be optimised: for example, samples could be sponged right after the biopsy to make sure that there is no blood that would skew the results (by increasing the apparent concentration of *Drug A* in the biopsy for example). Despite these variations, the CVs remained acceptable and data were exploitable.

#### **5.2.4 Were there any relations between skin distribution results safety and efficacy objectives of the study performed in psoriatic patients**

Local safety assessment (primary objective of the study) and skin distribution results:



During the study, six patients stopped the applications on one or several sites (of which five patients were stopped permanently and did not have any skin sampling) because of safety issues such as inflammation, oozing on/ or around the application sites (data not shown). A post-hoc analysis evaluated whether there was a correlation between subjects with high total skin concentration and safety issues. It appeared that only one of the subjects who had safety issues was sampled. The patient n°4 (see *Figure 34b*) was sampled on the zone treated with *Drug A* 5% and was also the one who had the highest total skin concentration, that is:  $\approx 269.10 \mu\text{g}/\text{cm}^2$  (at least twice as much as any other patients' results, See *Appendix D*). However, this single observation is not strong enough to support the hypothesis of a correlation between the quantity of drug that penetrates and local safety issues.

#### Efficacy assessment (second objective of the study) and skin distribution results:

Skin distribution results may support efficacy assessment as these measurements provide proof of exposure. For instance, thanks to the skin biopsy results, we do know that *Drug A* was present in the epidermis and dermis and was potentially bioavailable at the target of interest. What we do not know is its mechanism of action in the skin and whether the concentration was high enough to have a therapeutic effect. For instance, this question could be elucidated in PK/PD studies, where biopsies would be performed before and after treatment, in order to measure any variation of a target related biomarker. This was not the case in this study, where no PD assessment was done.

Unfortunately, in this study, the investigational compound did not show any clinical effect compared to the vehicle (although the active comparator 'calcipotriol' did, data not shown). This observation was then completed with skin distribution measurements, which showed that the molecule was actually present in the appropriate skin layers. Thus, it suggests that the new compound is not worthwhile for the treatment of psoriasis or that the dosage and/or formulation should be reviewed in order to obtain higher levels on the target site. The latter is unlikely since *Drug A* was formulated with the highest dosage possible and with the formulation that conferred the best penetration profile.

The early Go-NoGo decision whether to continue the development of *Drug A* in psoriasis was based on this PoC study, taking into consideration efficacy scoring and safety evaluation, but also the dermal PK results.

The perspective and benefit of performing PK measurements in clinical trials is multiple. Specific studies focusing on PK or PK/ PD measurement should be conducted more often and standardised in order to set-up a robust database with results that are comparable. Such studies should have PK or PK/ PD as their principal objective in order to focus on the dermal measurement and therefore optimise the procedure and plan appropriate sample size for more robust interpretation. For instance, in the psoriasis study, the expected number of samples was ten while only seven were available and analysed. Depending on the number and location of the biopsies, subjects do not seem to be that reluctant to the procedure, especially psoriatic patients whose biopsy is done on a psoriatic plaque, where healing is better (hyperkeratinisation) than on normal skin and where skin appearance is momentarily 'modified'. As clinical trial centres are getting used to performing biopsies in routine, patients should be more prone to accept this procedure, as far as biopsies do not leave any long-term scars (small biopsies: 2 to 4mm diameter) and are performed by well-trained staff under standardized conditions.

These preliminary results were useful as a first approach of skin distribution measurements and gave perspectives for further studies and optimised processes. This will help to get robust information for Go/ NoGo decision process in early development.

### 5.3. Overall cross analysis

#### 5.3.1 Data comparison

This overall cross analysis will compare the *in-vitro* liberation-penetration study performed on excised healthy human skin *versus* clinical studies performed on healthy volunteers and psoriatic patients.

According to *Franz T.J.*, the *in-vitro* finite dose model using excised human skin has proven to be a "*remarkably accurate representation of living man for assessing*

*topical pharmacokinetics*” (Franz, T.J. & al., 2009). For instance, having information on the investigational drug distribution in the different skin layers contributed to the choice of the right formulation before going to the clinic. The objective here is to compare the *in-vitro* skin distribution data of *Drug A* with clinical data obtained in ‘similar’ conditions. Similarities between the results would favour the use of this *in-vitro* study to predict human skin penetration and give early information on the concentration of the drug substance in the target tissue. A further perspective of this *ex-vivo* model would be to use psoriatic rather than healthy skin. This is quite challenging since the supply of ‘normal’ skin is already difficult.

The following graph illustrates the percentage (%) of the penetrated dose recovered in the SC and the (E+D) layers for the three studies (*Figure 39*).

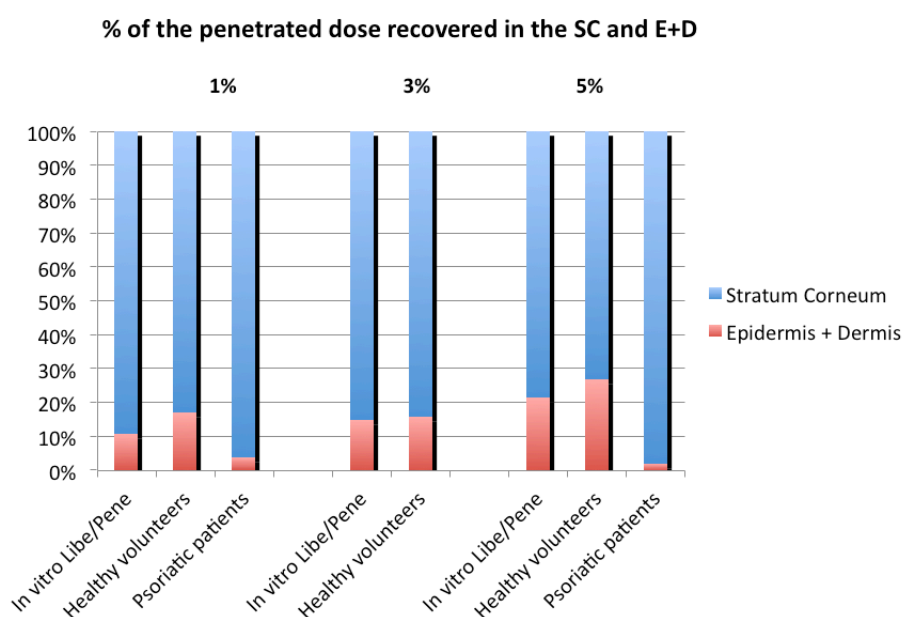
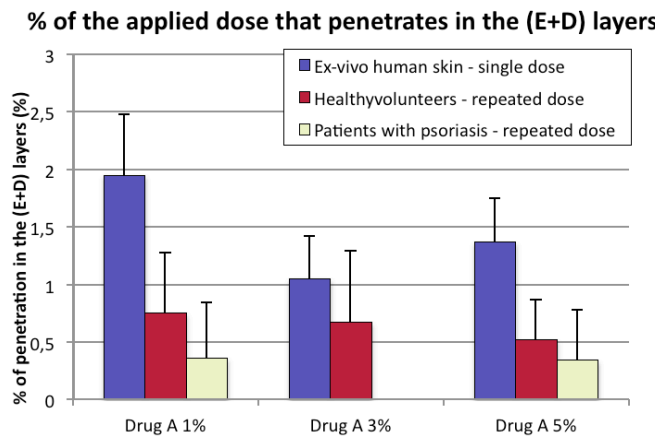


Figure n°39 | **The percentage of the penetrated dose recovered in the SC and (E+D) in the three studies, according to Drug A dosage. Adapted from Galderma documentation**

In all studies, the major penetrated amount of *Drug A* is retrieved in the Stratum Corneum rather than in the deeper layers. The profile of penetration is closer between the *in-vitro* study and healthy volunteers than between healthy volunteers and psoriatic patients (*Figures 39 & 41 + Appendices E & F for values*).

As the site of action is located in the (E+D), we will now focus on biopsy data (*Figures 40a & b*).

a.



b.

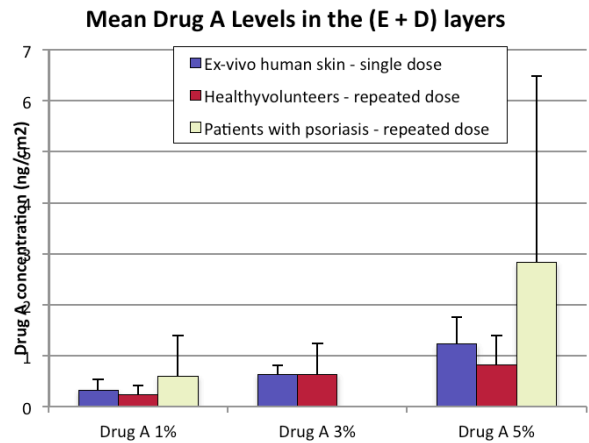


Figure n°40 | **a.** Mean % of the applied dose that penetrates in the (E + D) layers (Biopsy). **b.** Mean Drug A levels in the (E + D) layers (Biopsy), 24-hours after last applications. Adapted from Galderma documentation

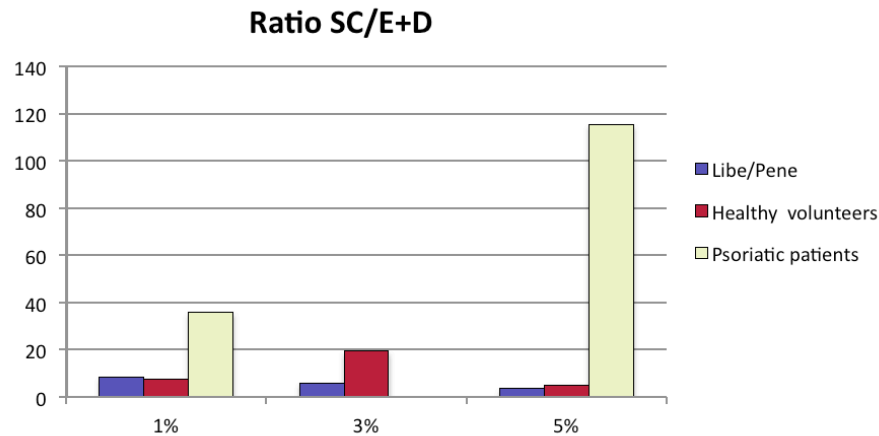


Figure n°41 | **Ratio between SC and (E+D) Drug A concentration in excised human skin, healthy volunteers, psoriatic patients, depending on Drug A dosage.** Adapted from Galderma documentation.

A few observations can be made on the *Figure 40a*, which shows the % of the applied dose that penetrated the (E + D) layers in the three models:

- Although the absolute concentration of penetrated drug in the (E+D) increased with *Drug A* dosage (*Figure 36b*), the % of the applied dose that penetrated in the (E+D) had a tendency to decrease with higher dosages, except for psoriatic patients ( $\approx 0.35\%$  of the applied dose for both *Drug A* dosages) (*Figure 40a*).
- Although the results are different for each study, the mean percentages of penetration were in the same range: all of them stand between  $[0.34 - 1.95\%]$  (*Figure 40a*).

- This analysis gives information on the difference of penetration according to the type of skin, with this specific drug substance in its specific formulation. The ranking of these models would be: *Ex-vivo* human skin (that overestimates skin penetration) > Healthy human skin > Psoriatic human skin (that confers to lowest penetration).

*Figure 40b* shows higher mean levels of *Drug A* in the (E+D) of human psoriatic skin because the quantity applied in total was approximately five times higher than for the other type of skin (833µg *versus* 160 µg for the healthy human skin and 196 µg for *ex-vivo* human skin). However, *Drug A* level in the (E+D) in psoriatic skin was not five times higher than the level found in healthy skin (*see Table XIV, Figure 36b*).

The mean concentration of *Drug A* 5% in psoriatic skin (*i.e.* 2.83µg/cm<sup>2</sup>) was particularly high because one patient had a high concentration value compared to the others, leading to a consequent increase of the mean level value (represented by the important error bar).

All the observations made on these graphs are exploratory and have to be confirmed in further PK studies. Meanwhile, they may help optimising PK measurements protocol in further studies.

### **5.3.2 Does liberation-penetration study reflect clinical situations in healthy volunteers and psoriatic patients treated with Drug A?**

#### Similarities and discrepancies between designs:

The key elements to ensure the success of *Franz* cells in predicting *in-vivo* bioavailability and percutaneous absorption pharmacokinetics (Raney, S. & al., 2008; Franz, T.J. & al., 2009) have been followed as closely as possible. For instance:

- There was an appropriate duplication in the *in-vitro* study of the essential physical parameters that significantly impact the absorption process in clinical condition (including temperature, humidity and suitable receptor solution).
- The technicians were fully trained in the use of the *Franz's* Cell model; and

- Had a good understanding of the penetrant's chemical characteristics in relation to skin physiology, formulation design, and diffusion process (not detailed here).
- There was attempt to duplicate clinical use conditions in the *in-vitro* study but several major discrepancies remained between designs:
  - Same formulation of *Drug A*
  - Non-occlusive conditions
  - Dose amount of product applied:  $4\mu\text{L}$  in the *in-vitro* study,  $10\mu\text{L}$  in healthy volunteers and  $50\mu\text{L}$  in psoriatic patients.
  - Same skin sampling protocol in the clinical studies. Tape stripping was also performed in the *in-vitro* study but skin biopsy was not needed.
  - Exposure durations and number of applications were almost similar for clinical studies (14 applications in 18 days for healthy volunteers and 18 applications in 21 days for psoriatic patients). The *in-vitro* study consisted in a single application followed by 16-hours of incubation (*See Table XIV*).
  - The location of skin samples were different for all models:
    - *In-vitro* study: 2 mammary and 1 abdominal skin donors
    - Healthy volunteers study: upper back
    - Psoriatic patients: arms, legs or trunk
  - The type of skin was different in each model:
    - *In-vitro* model: skin samples from donors who underwent skin surgery, certainly involving stretch marks
    - Healthy volunteers: "normal" skin
    - Psoriatic patients: pathological skin

As penetration profile may be influenced by skin structure and thickness, different sampling location in addition to 'not completely' harmonised protocols above-mentioned limit the analysis.

#### In-vitro model versus clinical situation:

##### ***Skin distribution:***

- *Drug A* was mainly recovered in Stratum Corneum, with:
  - 73% and 78% of *Drug A* 5% penetrated dose recovered in the SC in healthy volunteers and excised human skin respectively (*Figure 39*),

representing a *Drug A* concentration approximately 3.5 and 5-times higher in SC than (E+D) (*Figure 41*).

- 98% of *Drug A* 5% penetrated dose recovered in the SC in psoriatic patients, representing a *Drug A* concentration approximately 115-times higher in the SC than the (E+D).
- ⇒ Similar skin distribution of *in-vitro* excised human skin and *in-vivo* normal skin (healthy volunteers) in terms of percentage of the penetrated dose in the SC and the (E+D).

#### ***Dose proportionality:***

Despite high coefficient of variability, dose proportionality was observed in all models for the (E+D) *Drug A* concentrations, but not for the SC's ones. This may be explained by a saturation effect of the SC, inter-individual variability and variability related to the procedure

#### ***Skin penetration profile:***

- The *in-vitro* model overestimates skin penetration:
  - The percentage of the applied dose that penetrates in the SC is higher *in-vitro* than in healthy volunteers but not in psoriatic patients treated with *Drug A* 5% (*See Table XIIIb, section 5.1*): saturation effect with high *Drug A* dosage found in healthy volunteers and *in-vitro* model but not in psoriatic patients? (Modified histology of psoriatic SC, study conditions, low number of samples...)
  - The percentage of the applied dose that penetrates in the (E+D) is higher *in-vitro* than in clinical conditions
- ⇒ Conservative approach for safety

#### **Predictability of the *in-vitro* model:**

Despite the discrepancies between experimental conditions (mainly the number of applications and the quantity applied at each application, depending on the model), clinical study data and *in-vitro* results are in the same scale, especially in the target site (E+D), where dose proportionality was conferred for all models (*Figure 40b*). Besides, the *in-vitro* liberation-penetration study has a tendency to overestimate skin penetration of *Drug A* (except *Drug A* penetration in the SC, in psoriatic patients).

*In-vitro* data versus healthy volunteers study data: The *in-vitro* study reflected skin distribution profile (% of the penetrated dose recovered in the different skin layers) as well as dose proportionality of the (E+D) healthy volunteers' skin. However, it may overestimate skin penetration in normal skin. This should be confirmed in another study since the quantity of *Drug A* applied was different in the two models (2mg/cm<sup>2</sup> versus 3.2mg/cm<sup>2</sup>).

*In-vitro* data versus psoriatic patients study data: The *in-vivo* data obtained from psoriatic patients were not predicted by the *in-vitro* data, neither in the skin distribution profile, nor in the skin penetration profile. Indeed, the histological characteristics of psoriasis definitively have an impact on drug penetration, for this specific molecule and formulation, as observed throughout this cross analysis.

Finally, we might not be mistaken to say that the penetration is favoured in *ex-vivo* human skin compared to viable human skin (healthy or psoriatic subjects). However, it must be kept in mind that a non-viable skin model is obviously limited in its ability to accurately reflect a viable organ as the skin, notably in terms of skin metabolism, lack of vasculature etc... Moreover, it is challenging to have complete harmonised protocols.

In my opinion, getting PK preliminary results by conducting such studies, as well as comparing and potentially establishing correlations between models is a big step forward in order to provide more robust decision earlier in the development of a new drug. However, we must always keep in mind that the results and interpretation given for one drug substance and formulation may not be transposable for another compound. With knowledge acquisition and expertise from dedicated studies to skin distribution measurements with strictly similar designs, we may some day highlight general trends that will comfort the choice of the compound that confers the most favourable penetration of the drug substance and help the early Go/NoGo decision. Meanwhile, although non-clinical models provide essential preliminary information, we must remember that the best model to test anti-psoriatic agents remains patients with psoriasis.



## CONCLUSION -

Many questions have been addressed in this thesis. First we have seen some of the criteria that must be taken into consideration in Research & Development of new anti-psoriasis topical agents. Such criteria are the complex pathogenesis and pathophysiology of the disease, the drug characteristics required to penetrate through the skin up to the target site and the 'relative' predictability of non-clinical models in terms of pharmacodynamic, pharmacokinetic and safety properties.

Translational bidirectional flows of information between research and clinical settings offer an essential contribution to improve the screening of new topical treatments for psoriasis, early in development. Indeed, developing relevant non-clinical models (such as xenotransplantation of human psoriatic skin onto immunodeficient mice) and exploratory clinical trials (such as proof-of-concept studies using the modified *Dumas-Scholtz* design associated with several exploratory endpoints) are of great promise to select promising or terminate unpromising drugs earlier in the development.

The translational approach in this thesis consisted in performing a cross analysis that compares skin distribution results of a potential anti-psoriatic *Drug A* topical agent in non-clinical (*in-vitro* excised human skin) and clinical settings (healthy volunteers and psoriatic patients). The objectives of comparing such experimental results was to (1) assess the impact of psoriasis pathology on *Drug A* skin penetration by comparing healthy volunteers and psoriatic patients' data and to (2) evaluate the ability of *in-vitro* liberation-penetration studies to predict human skin penetration of *Drug A* in clinical settings. Skin samplings were performed with complementary dermal evaluation tools: tape stripping to collect the *Stratum Corneum* layers and skin biopsy to collect the *Epidermis* and *Dermis* together. The results showed that *Drug A* reached its target tissues (i.e. Epidermis and Dermis layers) in all models but the penetration profile was at some point different between models.

A quite similar *Drug A* skin distribution was found in *in-vitro* excised human skin and *in-vivo* normal skin (healthy volunteers) in terms of percentage of the penetrated

dose in the SC and the (E+D), with respectively 73 and 78% of *Drug A* 5% penetrated dose recovered in the SC. However, the *in-vitro* model overestimated skin penetration, meaning that the percentage of the applied dose that penetrated through the skin was higher *in-vitro* than in healthy volunteers.

Skin distribution was different in psoriatic patients with 98% of *Drug A* 5% penetrated dose found in the SC. Compared with psoriatic skin, the *in-vitro* model overestimated skin penetration in the (E+D) but not in the SC. Psoriasis histological skin features and/ or the high quantity applied might explain these results.

Dose proportionality was observed in all models in the (E+D), but not in the SC (see Table XIIIa). The latter may be explained by a saturation effect of the SC with high *Drug A* dosage, inter-individual variability and variability related to the tape stripping sampling procedure.

The interpretation of this cross analysis results is limited by discrepancies in the three designs, such as the *Drug A* frequency of application, the quantity of drug applied, the location of the zones of application. Moreover, the high coefficient of variation can be explained by the low number of skin samples, inter & intra-individual variability and finally by the variations related to the sampling procedure and bioanalysis.

Psoriasis pathology definitely impacts drug penetration through the skin. This is mainly due to histological differences found in psoriatic skin. For instance, the SC is thicker in psoriatic skin than normal skin, which may confer a reservoir effect of the SC. Moreover, the psoriatic compact pattern of the SC, in opposition to the basket woven pattern found in normal skin, makes drug penetration more difficult. As suggested by the results and according to the histology of psoriasis, drug penetration seems to be lower in psoriatic skin than in normal skin (healthy volunteers).

Although *in-vitro* and the exploratory clinical results were in the same scale, it is not possible to set definite conclusion on the ability of this *in-vitro* study to reflect humans for the reasons explained above. We may say that *Drug A* penetrates more *in-vitro* than *in vivo*. However, this observation only stands for the *drug substance A* and its specific formulation. Penetration profiles could be very different with another

molecule and/or another formulation. Moreover, it must be kept in mind that a non-viable skin model is obviously limited in its ability to accurately reflect a viable organ like the skin, notably in terms of skin metabolism, lack of vasculature etc. Moreover, it is challenging to have completely harmonised protocols that would allow relevant comparisons.

Bidirectional flows of exploratory information between research and clinical activities are very important for a translational medicine approach. In our case, it may help to (1) refine PK *in-vitro* screening models in predicting human skin penetration of drugs, (2) evaluate the relevance of human pharmacological surrogate model (healthy volunteers), (3) to refine and standardise sampling tools (tape-stripping and skin biopsy), and potentially (4) to get a better understanding of the disease as well as to identify new biomarkers. Indeed, developing non-clinical models that can predict and reflect the PK human situation would definitely be handy in early development, especially for a disease like psoriasis that is unique to human. Meanwhile, an option to obtain more robust early skin penetration results in clinic is to conduct studies whose principal objective would be skin sample measurements using well standardised skin sampling techniques in order to be in the best condition for relevant comparison.

The overall objective of such translational approach is eventually to make confident and robust screening decision as early as possible in the development of new anti-psoriatic drugs. For instance, the early skin distribution results in psoriatic patients, associated with the observed clinical lack of efficacy (compared to the positive comparator), supported the No Go decision of the *Drug A* project in the psoriatic indication. Terminating unpromising candidates earlier would therefore enable sponsors to re-direct R&D resources to more promising drug development programs.

## - CONCLUSION (français) -

THESE SOUTENUE PAR: *Aline SCHOLLER*

TITRE: **EARLY SCREENING IN DERMATOLOGY USING TRANSLATIONAL MEDICINE: APPLICATION FOR A NEW TOPICAL AGENT IN THE TREATMENT OF PSORIASIS**

Un grand nombre de questions ont été abordées dans cette thèse. En premier lieu, nous avons vu les critères devant être pris en compte lors de la recherche et du développement de traitements topiques du psoriasis. Parmi ces critères, on trouve l'étiologie et la physiopathologie de cette maladie dermatologique, les caractéristiques de la molécule et la formulation requise pour pénétrer la peau jusqu'au site d'action. La prédictibilité relative des modèles non-cliniques, en termes de pharmacodynamie, pharmacocinétiques et de tolérance, est aussi à prendre en compte. Cela est d'autant plus vrai que le psoriasis est une maladie affectant uniquement l'homme.

Le transfert de connaissances et d'informations entre la recherche et le développement clinique (et vice versa) facilitera la sélection de nouveaux traitements topiques du psoriasis, et ce, dès les premières phases de développement. Le développement de modèles non-cliniques pertinents (tel que la xenotransplantation de peau humaine atteinte de psoriasis sur le flanc de souris immunodéprimées) et des essais cliniques exploratoires (tel que les études de « Preuve-de-Concept » utilisant le design *Dumas-Scholtz* modifié) sont des méthodes prometteuses pour la sélection ou l'arrêt précoce d'un projet de développement de médicament.

L'approche translationnelle envisagée ici consiste à réaliser une étude transversale comparant, pour une même molécule candidate, les résultats de distribution dans la peau en condition non-clinique (étude *in-vitro* avec de la peau humaine excisée) et en clinique (volontaires sains et patients atteints de psoriasis). L'objectif de cette analyse est (1) d'évaluer l'impact de la maladie sur la pénétration de la *molécule A*, en comparant les données du volontaire sain à celles du patient ; et (2) d'évaluer la capacité des études de libération-pénétration *in-vitro* à prédire la pénétration dans la peau de la *molécule A* en clinique. Les échantillons de peau ont été prélevés à l'aide de deux méthodes complémentaires : le « tape stripping » pour recueillir la couche cornée et la biopsie de peau pour recueillir l'épiderme et le derme. Les résultats d'analyse ont montré que la *molécule A* avait atteint son site d'action (épiderme + derme) dans tous les modèles. Cependant le profil de pénétration était différent entre chacun de ces modèles.

Le profil de distribution de la *molécule A* dans les différentes couches de la peau *in-vitro* était proche celui trouvé *in-vivo* chez des volontaires sains. En effet, 73% et 78% de

la *molécule A 5%* pénétrée s'était accumulé au niveau de la couche cornée, *in-vivo* et *in-vitro* respectivement. Cependant, le modèle *in-vitro* surestimait toutes les concentrations dans les différentes couches de la peau.

Le profil de distribution était différent chez les patients atteints de psoriasis avec 98% de *molécule A 5%* pénétrée accumulée au niveau de la couche cornée. Par rapport à la peau psoriasique, le modèle *in-vitro* surestimait la pénétration au niveau de l'épiderme + derme mais pas au niveau de la couche cornée. Les propriétés histologiques de la peau psoriasique mais aussi la quantité plus importante du *produit A* appliqué dans l'étude psoriasis permettent certainement d'expliquer ces résultats.

Une dose proportionnalité a été statistiquement observée au niveau de l'épiderme + derme pour tous les modèles (non-cliniques et cliniques). Il n'y a pas eu de tests statistiques réalisés sur les concentrations trouvées dans la couche cornée, mais d'après l'analyse descriptive, il semble qu'il n'y avait pas de dose proportionnalité au niveau de la couche cornée, et ce, quel que soit le modèle. Cela pourrait être expliqué par un phénomène de saturation de la couche cornée (lorsqu'une concentration plus élevée du *produit A* est appliquée), par les variabilités inter & intra-individuelles et celles liées à la procédure d'échantillonnage.

L'interprétation des résultats de cette analyse transversale est tout de même limitée par les différences retrouvées entre les trois protocoles. Parmi ces différences, on retrouve la quantité de dose appliquée, la fréquence d'application et la localisation des zones d'application. De plus, les coefficients de variation élevés sont, entre autre, dus au faible nombre d'échantillons (en particulier dans l'étude psoriasis), aux variabilités inter & intra-individuelles ainsi qu'aux variabilités liées à la procédure d'échantillonnage (tape stripping et biopsie).

L'impact de la pathologie sur la pénétration de la *molécule A* à travers la peau semble évident. Ceci s'explique principalement par les différences histologiques entre une peau psoriasique et une peau normale. En effet, sa couche cornée plus épaisse que celle d'une peau normale semble lui conférer un effet réservoir. De plus, les cellules (keratinocytes possédant encore leur noyau) en jonctions serrées au niveau de la couche cornée d'une plaque de psoriasis, en opposition aux cellules mortes (keratinocytes sans noyau) plus espacées de la peau normale, rendent la pénétration de molécules plus difficile. Comme suggéré par les résultats, la pénétration jusqu'au site d'action semble être diminuée sur une peau psoriasique par rapport à celle d'une peau normale.

Bien que les concentrations absolues trouvées *in-vitro* et *in-vivo* soient dans la même fourchette, il n'est pas possible d'établir de conclusion définitive sur la capacité de l'étude *in-vitro* à refléter l'homme pour les raisons expliquées précédemment. On peut tout de même dire que la *molécule A* pénètre plus *in-vitro* que *in-vivo*. Il est à noter que cette observation n'est valable que pour cette *molécule A* avec cette formulation. Le profil de pénétration pourrait être très différent pour une autre molécule et une autre formulation. De plus, il faut garder en tête qu'un modèle de peau non-viable est forcément limité dans sa capacité à refléter un organe viable tel que la peau, notamment en terme de métabolisation par la peau, du manque de vascularisation etc. De plus, il est difficile d'harmoniser les protocoles d'études qui permettraient de comparer les résultats de façon pertinente.

Le transfert d'information entre la recherche et les activités cliniques est très important pour améliorer la sélection de molécule candidate en développement précoce. Dans notre cas, cette approche translationnelle peut aider pour (1) améliorer les modèles de sélection *in-vitro* grâce aux données obtenues chez l'homme, (2) évaluer la pertinence de modèles pharmacologiques humains (volontaires sains par rapport aux patients par exemple), (3) améliorer et standardiser les techniques d'échantillonnage (tape stripping et biopsie de la peau) et éventuellement (4) acquérir une meilleure compréhension de la maladie et identifier des biomarqueurs. En effet, développer des modèles non-cliniques pouvant prédire et refléter les conditions chez l'homme serait un grand progrès en développement clinique précoce, d'autant plus qu'il n'existe pas de bon modèle animal de cette pathologie. La réalisation d'études dédiées à l'évaluation de la pénétration d'une molécule candidate permettrait d'être dans les meilleures conditions pour faire des comparaisons plus pertinentes.

L'objectif primaire de cette approche translationnelle est finalement de prendre des décisions de Go/NoGo les plus robustes possibles, et ce, dès les premières phases du développement. Par exemple, les résultats de pénétration de la *molécule A* de l'étude psoriasis, associés au manque d'efficacité clinique par rapport au comparateur positif (calcipotriol), ont soutenu la décision de NoGo du projet *médicament A* dans le psoriasis. L'arrêt précoce de projets de développement de médicaments non prometteurs permet donc aux laboratoires pharmaceutiques de rediriger leurs ressources sur d'autres programmes aux résultats plus encourageants.

VU ET PERMIS D'IMPRIMER

Grenoble, le:

LE DOYEN

LE PRESIDENT DE LA THESE

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- APPENDICES -

Healthy Volunteers Data

Strip nb	Drug A 1%		Drug A 3%		Drug A 5%	
	Mean	SD	Mean	SD	Mean	SD
1 (Strip excès)	-	-	-	-	-	-
# 2-3	397,13	533,76	2850,06	2911,49	1154,28	2111,50
# 4-5	213,75	151,34	1550,67	1803,61	622,61	970,75
# 6-7	168,69	82,76	721,00	739,50	392,22	461,38
# 8-9	140,81	73,70	513,50	507,31	293,11	322,52
# 10-11	97,38	35,45	430,94	406,96	229,89	213,45
# 12-13	86,31	47,03	368,56	431,27	210,56	238,76
# 14-15	77,94	48,42	254,89	234,33	123,17	101,59
# 16-17	57,88	28,65	271,56	284,12	124,89	98,22
# 18-19-20	84,69	55,68	327,28	332,55	186,44	202,89
<b>Total SC (ng/cm<sup>2</sup>)</b>	1324,56	929,03	7288,44	7113,99	3337,17	4619,81
<b>Biopsy (ng/0,07cm<sup>2</sup>)</b>	16,99	11,66	45,04	42,16	57,78	39,54
<b>Biopsy (ng/cm<sup>2</sup>)</b>	242,68	166,62	643,49	602,34	825,40	564,87
<b>Total skin (ng/cm<sup>2</sup>)</b>	1567,24	956,48	7931,94	7212,30	4162,56	4789,84

Appendix A| Mean concentration and Standard Deviation values of tape-strips, total SC, biopsies (E+D) and Total Skin in healthy volunteers, according to Drug A dosage. Adapted from Galderma documentation.

Psoriatic Patients Data

Strip nb	Drug A 1%			Drug A 5%		
	Mean	SD	CV (%)	Mean	SD	CV (%)
1 (Strip excès)	-	-	-	-	-	-
2 (Strip 2-3)	4903,00	4138,59	84%	71170,00	36119,52	51%
3 (Strip 4-5)	2346,67	1205,04	51%	17749,67	7863,37	44%
4 (Strip 6-7)	2526,00	2996,05	119%	26127,50	36402,64	139%
5 (Strip 8-9)	1350,33	986,64	73%	20487,75	31657,86	155%
<b>Total SC (ng/cm<sup>2</sup>)</b>	11126,00	9322,97	84%	131097,50	89818,23	69%
<b>Biopsy (ng/0,07cm<sup>2</sup>)</b>	41,80	55,80	134%	198,38	255,36	129%
<b>Biopsy (ng/cm<sup>2</sup>)</b>	597,14	797,21	134%	2833,93	3647,97	129%
<b>Total skin (ng/cm<sup>2</sup>)</b>	11723,14	10120,16	86%	133931,43	93153,29	70%

Appendix B| Mean concentration, Standard Deviation and Coefficient of Variation values of tape-strips, total SC, (E+D) and Total Skin in psoriatic patients, according to Drug A dosage. Adapted from Galderma documentation

**Psoriatic Patients detailed Data**  
**Drug A 1%**

Strip nb/ Patient N°	12	2	5	Mean	SD	CV (%)
1 (Strip exces)	-	-	-	-	-	-
2 (Strip 2-3)	2138,00	2910,00	9661,00	4903,00	4138,59	84%
3 (Strip 4-5)	1545,50	1762,00	3732,50	2346,67	1205,04	51%
4 (Strip 6-7)	694,50	900,00	5983,50	2526,00	2996,05	119%
5 (Strip 8-9)	699,00	866,50	2485,50	1350,33	986,64	73%
<b>Total SC (ng/cm2)</b>	5077,00	6438,50	21862,50	11126,00	9322,97	84%
<b>Biopsy (ng/0,07cm2)</b>	6	13,3	106,1	41,80	55,80	134%
<b>Biopsy (ng/cm2)</b>	85,71	190,00	1515,71	597,14	797,21	134%
<b>Total skin (ng/cm2)</b>	5162,71	6628,50	23378,21	11723,14	10120,16	86%

Appendix C| Individual data source of **Drug A 1%** tape-strips, total SC, (E+D) and Total Skin in psoriatic patients.  
Adapted from Galderma documentation

**Psoriatic Patients detailed Data**  
**Drug A 5%**

Strip nb/ Patient N°	4	6	9	3	Mean	SD	CV (%)
Strip nb	-	-	-	-	-	-	-
1 (Strip exces)	112765,50	49348,00	33788,00	88778,50	71170,00	36119,52	51%
2 (Strip 2-3)	-	12075,00	14448,50	26725,50	17749,67	7863,37	44%
3 (Strip 4-5)	80214,00	5649,50	15067,50	3579,00	26127,50	36402,64	139%
4 (Strip 6-7)	67915,00	4591,00	6662,00	2783,00	20487,75	31657,86	155%
<b>Total SC (ng/cm2)</b>	260894,50	71663,50	69966,00	121866,00	131097,50	89818,23	69%
<b>Biopsy (ng/0,07cm2)</b>	573,8	45,5	144,3	29,9	198,38	255,36	129%
<b>Biopsy (ng/cm2)</b>	8197,14	650,00	2061,43	427,14	2833,93	3647,97	129%
<b>Total skin (ng/cm2)</b>	269091,64	72313,50	72027,43	122293,14	133931,43	93153,29	70%

Appendix D| Individual data source of **Drug A 5%** tape-strips, total SC, (E+D) and the Total Skin, in psoriatic patients.  
Adapted from Galderma documentation

Ratio SC/E+D	1%	3%	5%
Libe/Pene	8,24	5,73	3,65
Healthy volunteers	7,62	19,52	5,14
Psoriatic patients	35,85	-	115,33

Appendix E | **Ratio of the mean concentration in the Stratum Corneum and the Epidermis + Dermis in the three models, according to Drug A dosage.** Adapted from Galderma documentation

Skin distribution		Stratum Corneum	Epidermis + Dermis
1%	Libe/Pene	89,18	10,82
	Healthy volunteers	83,06	16,94
	Psoriatic patients	96,33	3,67
3%	Libe/Pene	85,13	14,87
	Healthy volunteers	84,38	15,62
5%	Libe/Pene	78,50	21,50
	Healthy volunteers	73,12	26,88
	Psoriatic patients	98,21	1,79

Appendix F | **Skin distribution: Percentage of the penetrated dose recovered in the SC and E+D in the three models, according to Drug A dosage.** Adapted from Galderma documentation

**- SERMENT DES APOTHICAIRES -**

*Faculté de Pharmacie de Grenoble*

# **Serment des Apothicaires**

*Je jure, en présence des maîtres de la faculté, des conseillers de l'ordre des pharmaciens et de mes condisciples:*

*D'honorer ceux qui m'ont instruit dans les préceptes de mon art et de leur témoigner ma reconnaissance en restant fidèle à leur enseignement.*

*D'exercer, dans l'intérêt de la santé publique, ma profession avec conscience et de respecter non seulement la législation en vigueur, mais aussi les règles de l'honneur, de la probité et du désintéressement.*

*De ne jamais oublier ma responsabilité et mes devoirs envers le malade et sa dignité humaine: en aucun cas je ne consentirai à utiliser mes connaissances et mon état pour corrompre mes mœurs et favoriser des actes criminels.*

*Que les hommes m'accordent leur estime si je suis fidèle à mes promesses. Que je sois couvert d'opprobre et méprisé de mes confrères si j'y manque.*

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**TITRE : EARLY SCREENING IN DERMATOLOGY USING TRANSLATIONAL MEDICINE:  
APPLICATION FOR A NEW TOPICAL AGENT IN THE TREATMENT OF PSORIASIS**

**- ABSTRACT -**

Psoriasis is a common, chronic inflammatory skin disorder characterised by keratinocytes hyperplasia and incomplete differentiation. It affects 0.6 to 4.8% of the general population. The various existing treatments are not always satisfying because of lack of efficacy and/or safety concerns. Streamlining clinical research for the development of new anti-psoriasis therapies consists in developing more pathophysiological-based molecule, relying on relevant non-clinical models and performing early clinical trials as early as possible since the best model remains the human. More than just accelerating drug development, such approaches should provide reliable information needed for the Go/NoGo decisions from the early development of a molecule. An application of a translational approach is presented in this thesis. A cross analysis was carried-out in order to compare penetration profiles of a *Drug A* in three different models: one non-clinical *in-vitro* model (excised human skin) and two clinical ones, healthy volunteers (human pharmacology model) and psoriatic patients (the target population). Among other things, these results supported a NoGo decision early in development, allowing a redirection of R&D resources to more promising drug development programs.

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Key words:

Psoriasis –Clinical development – Translational Medicine – topical agent –  
Model– *In-vitro* – Biopsy – skin distribution



**TITRE : EVALUATION CLINIQUE PRECOCE EN DERMATOLOGIE PAR LA MEDECINE  
TRANSLATIONNELLE: APPLICATION POUR UN NOUVEL AGENT TOPIQUE DANS LE  
TRAITEMENT DU PSORIASIS**

Le psoriasis est une pathologie chronique inflammatoire caractérisée par une hyperplasie et une différenciation incomplète des kératinocytes. Elle touche entre 0,6 et 4,8% de la population générale. Bien que nombreux et divers, les traitements existants ne sont pas toujours satisfaisants en terme d'efficacité et/ou de tolérance. Développer de nouvelles thérapies basées sur la physiopathologie de la maladie, utiliser des modèles non-cliniques plus pertinents et réaliser des essais cliniques précoces chez le patient, permettraient d'optimiser la recherche clinique dans le développement de nouvelles thérapies pour psoriasis. Ce genre d'approche translationnelle devrait permettre d'obtenir le maximum d'informations pertinentes nécessaires pour la prise de décision sur la viabilité d'un projet dès le début du développement. Une mise en application d'une telle approche est présentée dans cette thèse. La réalisation d'une analyse transversale comparant les profils de pénétration d'une *molécule A* dans trois modèles différents : un modèle *in-vitro* non-clinique (peau humaine excisée) et deux modèles cliniques (volontaires sains et patients atteint de psoriasis). Les résultats ont, entre autre, aidés à prendre la décision d'arrêter le projet. Ainsi, les ressources de ce projet 'non prometteur' peuvent alors être réinvesties dans d'autres programmes de développement plus encourageants.

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Mots clés :

Psoriasis –Développement Clinique – Médecine translationnelle – Traitements topiques –  
Modèles– *In-vitro* – Biopsie – Distribution cutanée

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