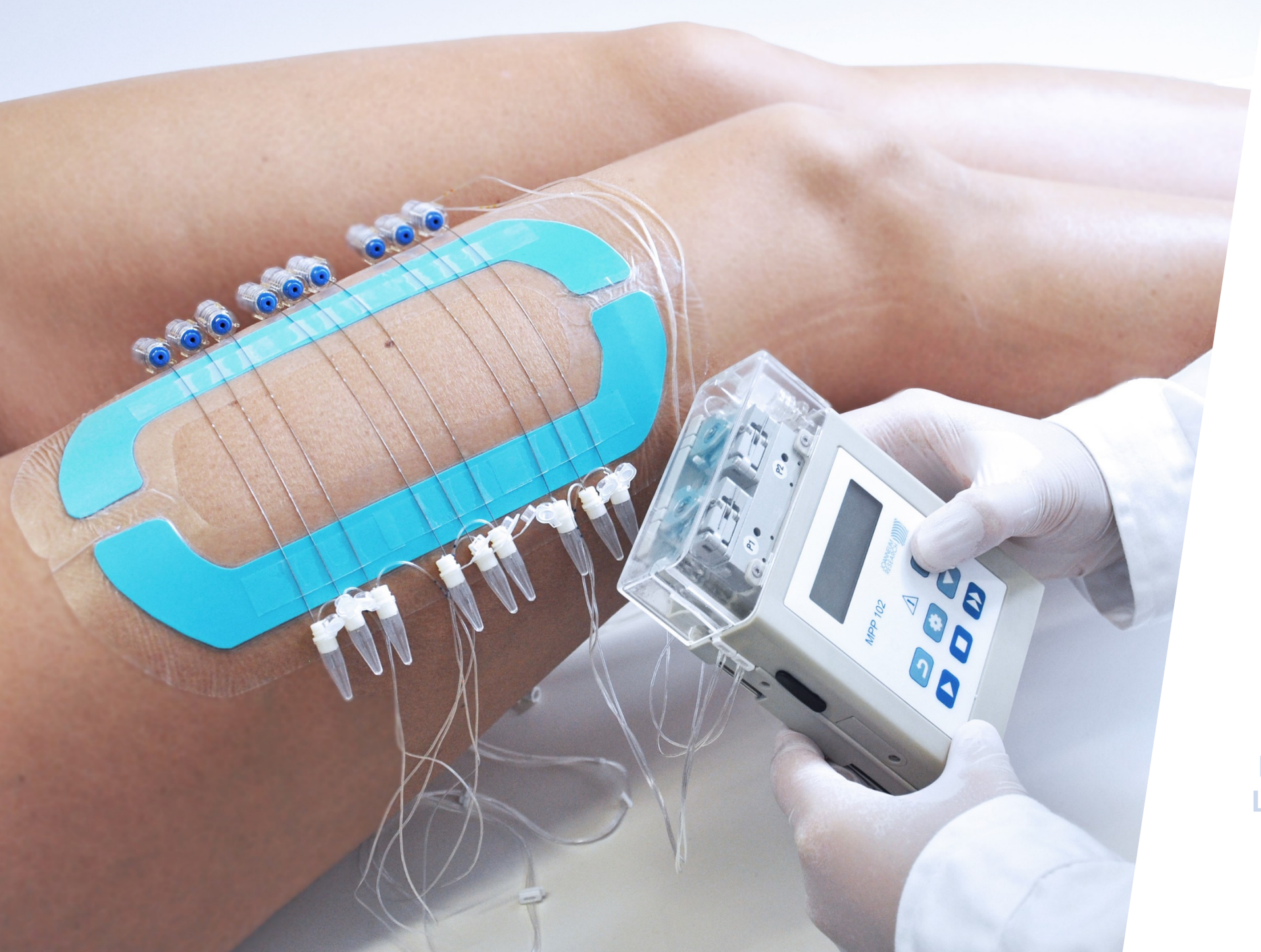

JOANNEUM RESEARCH HEALTH

Webinar

Informed decisions in dermal drug development with dOFM

November 10, 2020 3:00 pm
London / 10:00 am New York

dOFM

Who we are

Frank Sinner, PhD

Director JOANNEUM RESEARCH HEALTH

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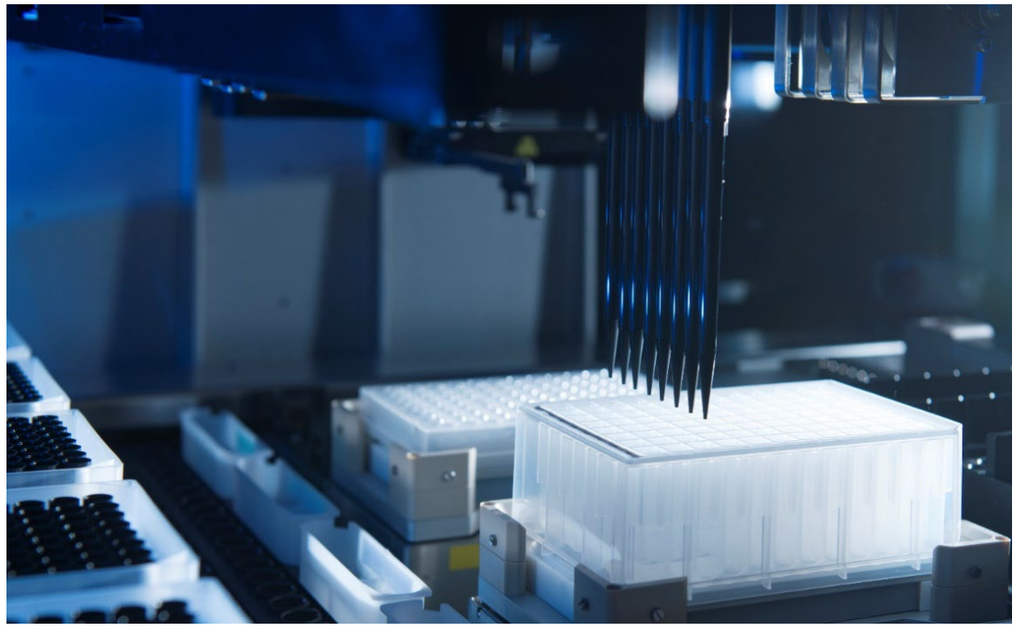
Thomas Birngruber, PhD

Group Leader OFM & Business Development

thomas.birngruber@joanneum.at

Who we are

Boutique CRO for research projects & drug development programs



We support your clinical and preclinical activities in the fields of diabetes, **dermatology** and metabolic research by providing:

- (Pre)clinical PK/PD/BE studies at the target tissue level
- In-vitro release testing (IVRT)
- Customized bioanalyses (PK, PD) – GLP certified lab
- Metabolomics
- Electronic data capture and data management
- Biostatistics
- Medical writing

Learn more about our CRO services at croservices.joanneum.at

Source: JOANNEUM RESEARCH

Key Learnings

How can dOFM improve your dermal drug development.

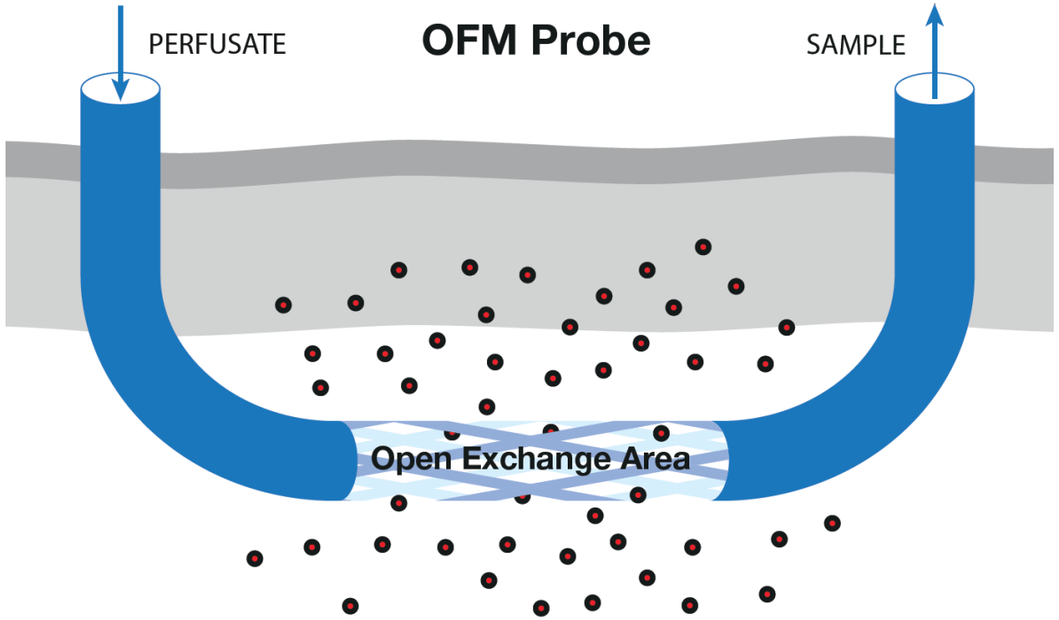
How to monitor local drug concentration in the dermis.

How to perform a head-to-head comparison in the same subject.

How to translate preclinical results directly into the clinical situation.

dOFM Working Principle

- Sampling of lipophilic substances [1,2], high molecular weight substances[3,4], immune cells, etc.
- Simultaneous/separated monitoring of PK and PD.
- Substances can be applied directly into the dermis via dOFM.
- Time-resolved monitoring for up to 48 h.
- Same dOFM setup in clinical, preclinical and ex-vivo studies.



Source: JOANNEUM RESEARCH

OFM samples represent diluted but unfiltered interstitial fluid.

[1] CP-17; logP 3.5 (Bodenlenz et al. 2016) [2] fentanyl; logP 4.5 (Holmgaard et al. 2011) [3] antibodies (Dragatin et al. 2016) [4] cytokines (Kolbinger et al. 2016)

Custom-Made Projects by Combining Models & Methods

dOFM Models

Ex-vivo

- Rat, pig, human tissue
- High throughput screening (fast and economical)
- No blood flow

Preclinical In-vivo

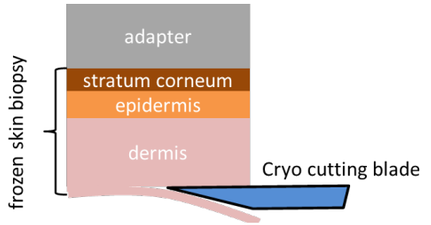
- Rat, pig
- High throughput screening (fast and economical)
- Blood flow

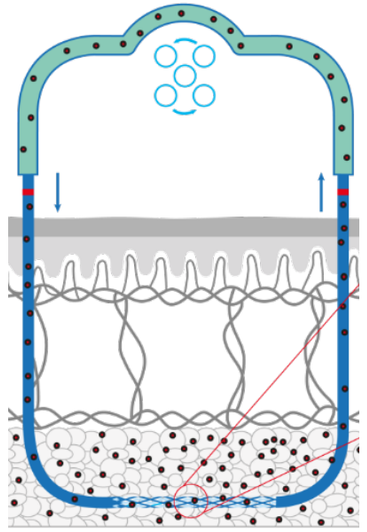
Clinical

- Highest relevance
- Long monitoring times (48 h)
- Multiple sampling locations

Complementary Methods

- Cryo-cut biopsy (depth-resolved)
- Blood sampling
- Absolute quantification
- Recirculation, microdosing
- Other technologies e.g. RAMAN, etc.

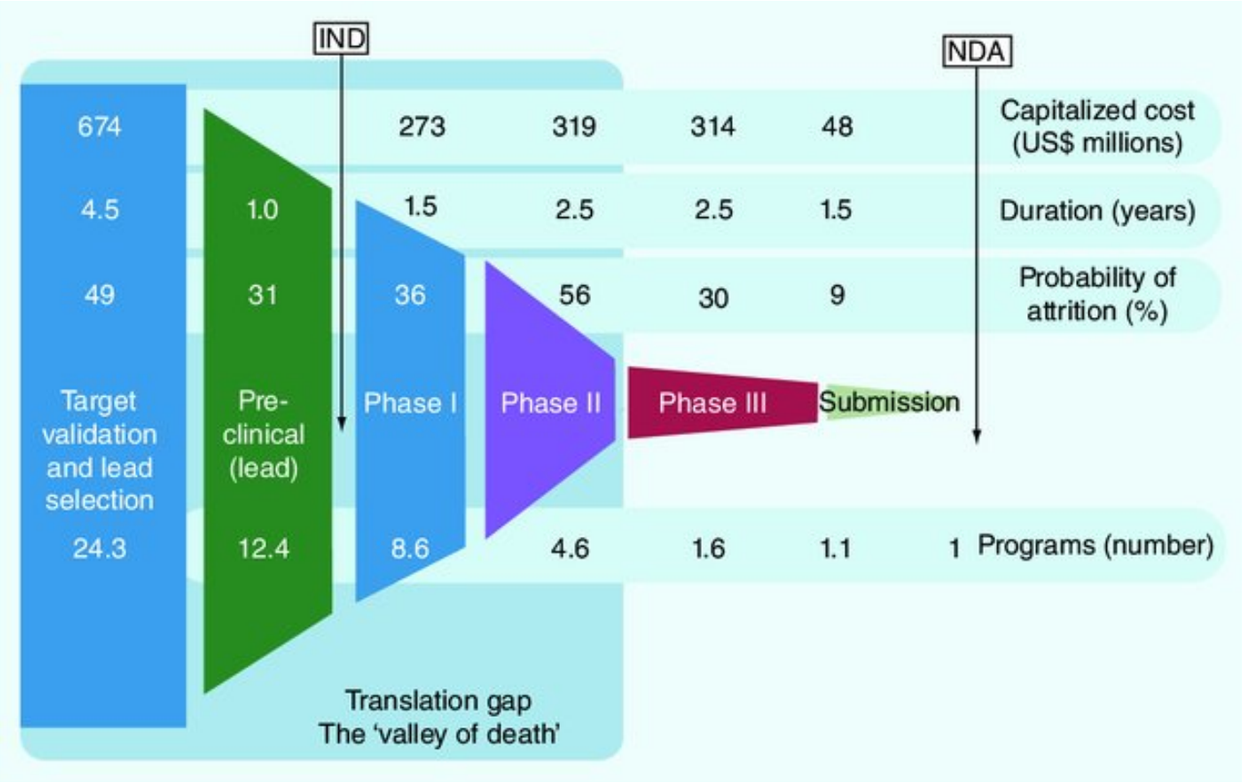




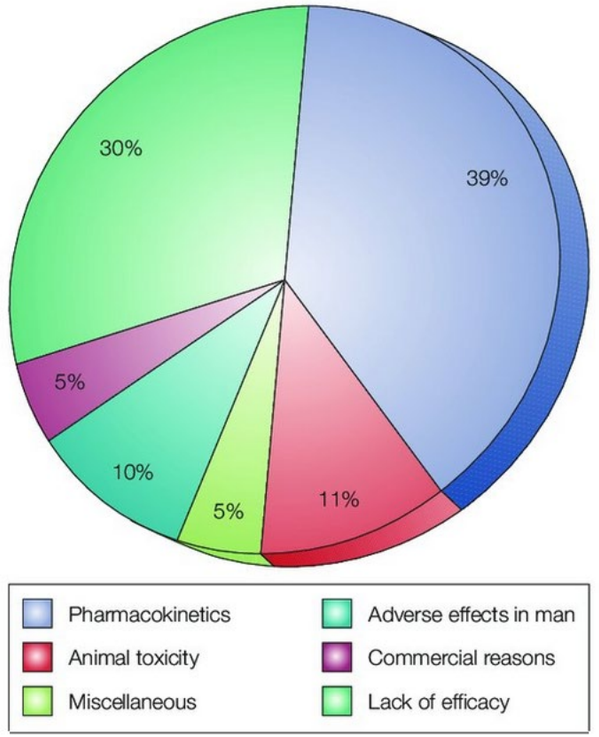
Source: JOANNEUM RESEARCH

Drug Development Process

Reasons for failure in drug development



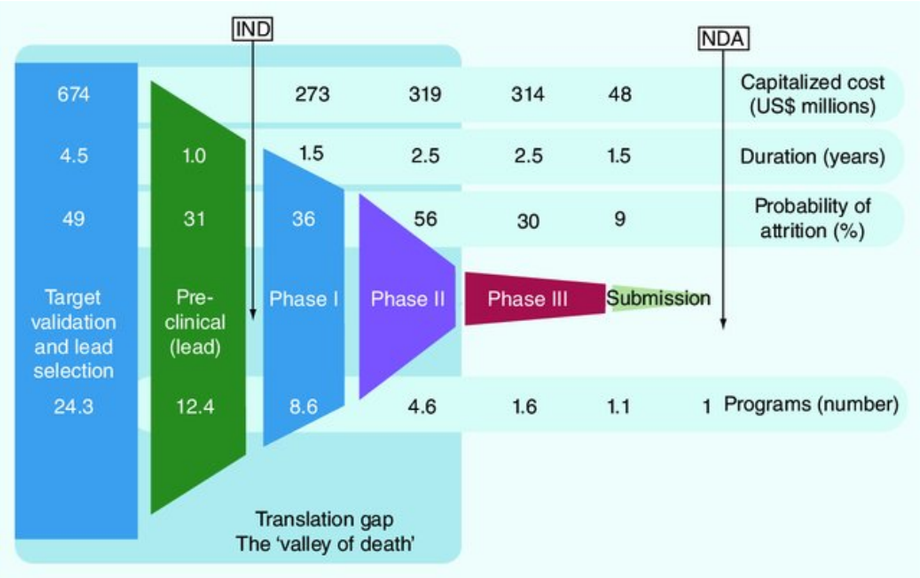
Source: Report: April 2013 Pharmaceutical Bioprocessing 1(1):29-50 DOI: 10.4155/pbp.13.3



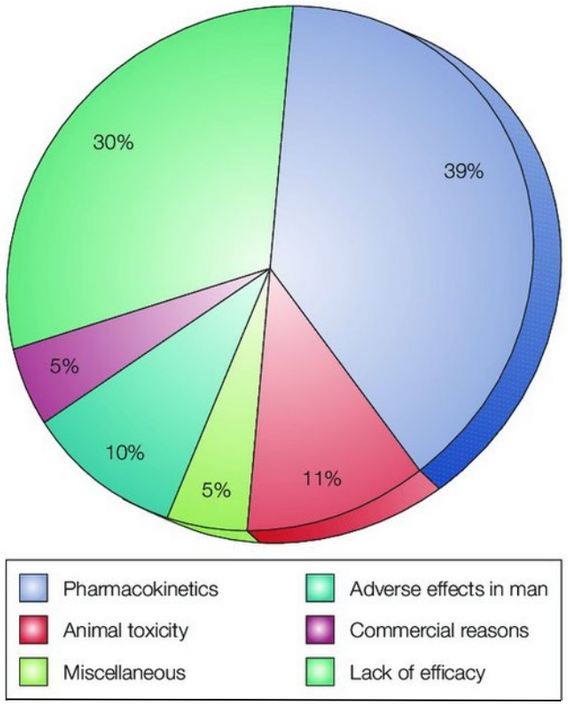
Source: Nature Reviews Drug Discovery (PMID: 12612645)

Drug Development Process

dOFM studies can reduce failure risk



Source: Report: April 2013 Pharmaceutical Bioprocessing 1(1):29-50
DOI: 10.4155/pbp.13.3



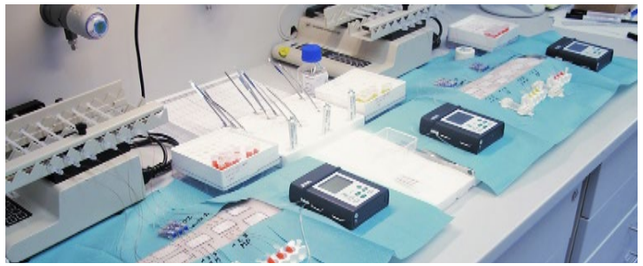
Source: Nature Reviews Drug Discovery (PMID: 12612645)

dOFM studies help to

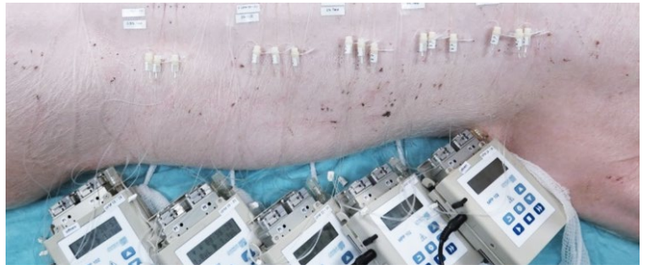
- reduce risks
- reduce costs
- save time

by creating high quality data at an early stage.

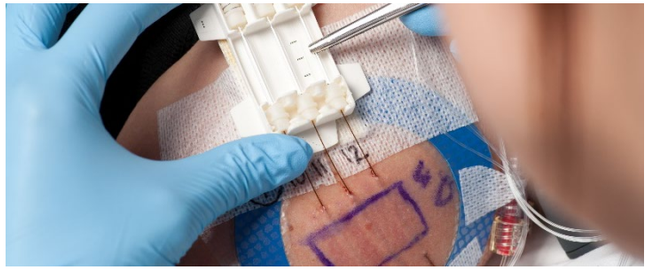
dOFM Models and Case Studies



Ex-Vivo Models and Case Study



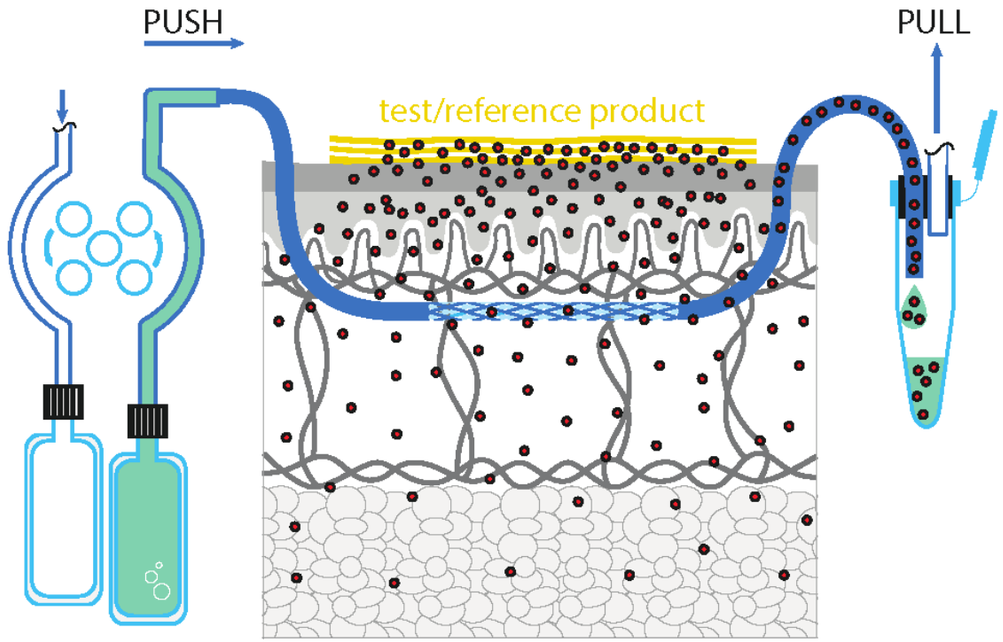
Preclinical Models and Case Study



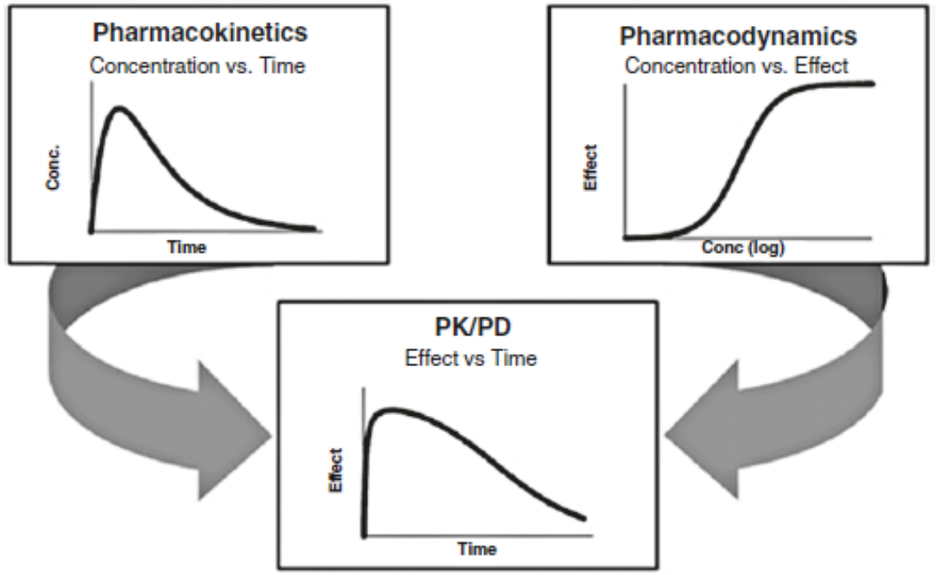
Clinical Setup & Case Studies

Drug Development Process

Continuous monitoring of PK and PD in the skin



Source: JOANNEUM RESEARCH



dOFM allows separate or simultaneous investigation of PK and PD in the target tissue.

Trottet, Maibach; 2017 “Dermal drug selection and development – An industrial perspective”

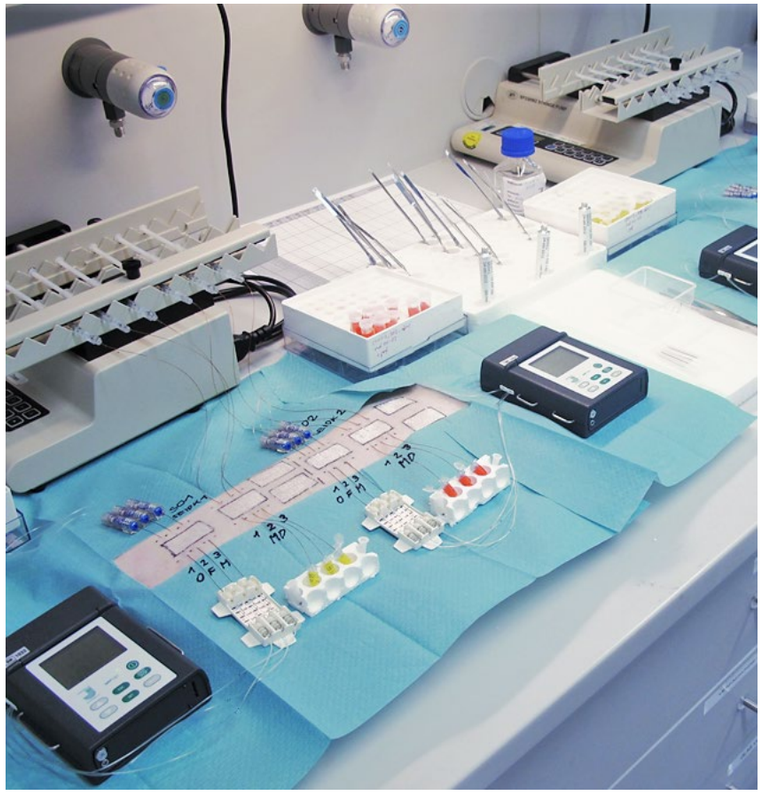
Source: JOANNEUM RESEARCH

Ex-Vivo Models & Case Study

Ex-Vivo Models

Economical set-up for PK comparison and drug stability

- **Available models:** pigs and excised human tissue (fresh)
- **Experimental duration:** up to 48 hours
- **Time resolution:** 5 min to hours (dependent on sample volume for analytics)
- **Application sites**: up to 30 sites with 3 dOFM probes each
- **OFM material:** same material as in clinical trials



Ex-Vivo Case Study: Drug Effect Prediction

Can dOFM predict drug effects in atopic dermatitis?

Overall study aim

Development of a topical drug for atopic dermatitis treatment (PD4 inhibitor) which has …

- high dermal API levels for drug effect (>EC50).
- low systemic concentration to reduce side effects (high systemic clearance).

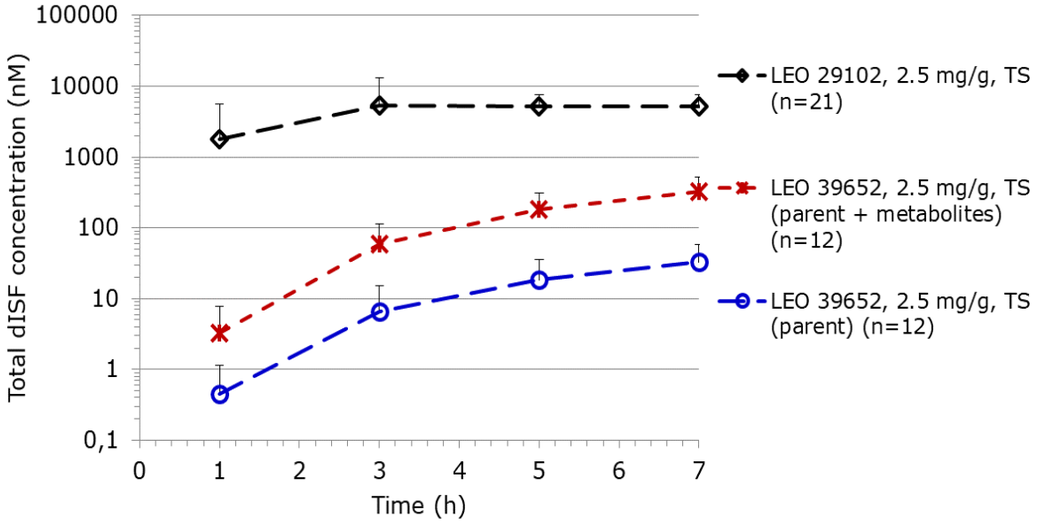
dOFM study aims

Ex-vivo dOFM investigations were preformed regarding

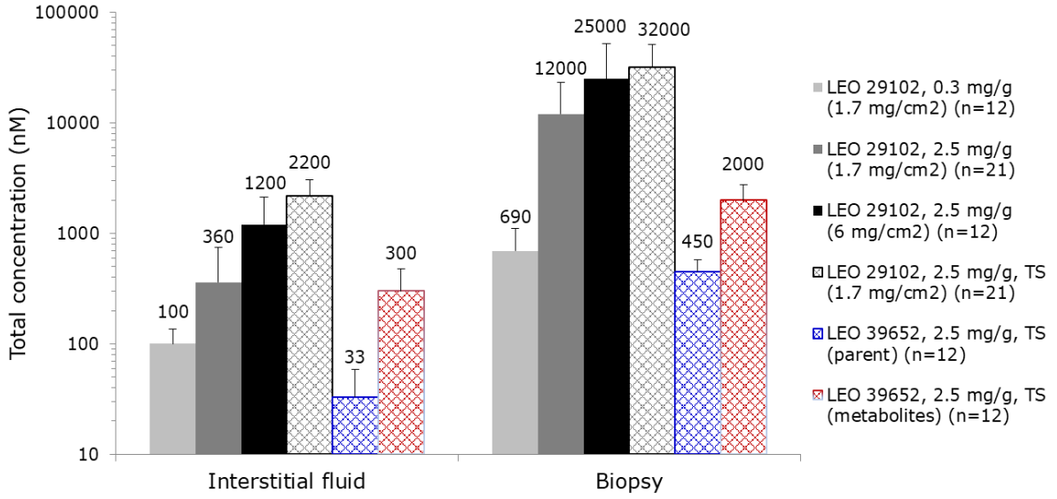
- PK across skin barrier,
- skin metabolism, and
- determination of free fraction in the dermis.

Ex-Vivo Case Study: Drug Effect Prediction

dOFM PK data show less variability compared to biopsies



dOFM PK profile of LEO 29102, LEO 39652 and the sum of LEO 39652 and its major metabolites in interstitial fluid (ISF).



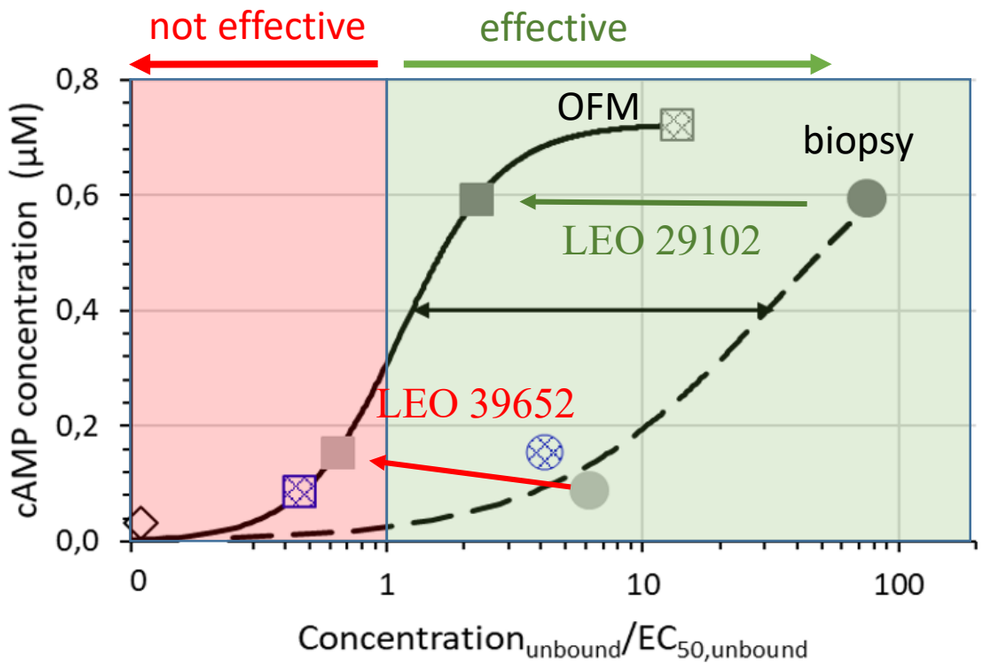
Total dISF and biopsy concentrations of LEO 29102, LEO 39652 and the metabolites of LEO 39652.

Biopsies show a higher standard deviation compared to dOFM samples due to high contamination in biopsies.

Sources: JOANNEUM RESEARCH

Ex-Vivo Case Study: Drug Effect Prediction

dOFM correctly predicted skin exposure



Compounds selected for clinical trial (based on biopsies)

- LEO 29102 : low MW, log D~3; protein binding 95%; EC50 ~60 nM
- LEO 39652 : low MW, log D~3; protein binding 98%; EC50 ~80 nM

Clinical trial showed

- clinical efficacy for LEO 29102.
- lack of efficacy for LEO 39652.

Falsely high skin exposure by biopsies vs. correct dOFM data.

Eirefelt S, et al. Evaluating Dermal Pharmacokinetic and Pharmacodynamic Effect of Soft Topical PDE4 Inhibitors: Open Flow Microperfusion and Skin Biopsies. Accepted in Pharm Res.

Source: JOANNEUM RESEARCH

Preclinical Models & Case Study

In-Vivo Preclinical Models

Economical set-up for in-vivo PK head-to-head comparisons

- **Available models:** pigs and rats
- **Experimental duration:** up to 14 hours
- **Application sites**: up to 14 sites with 3 dOFM probes each
- **Time resolution:** 5 min to hours (dependent on sample volume for analytics)
- **OFM material:** same material as in clinical trials

Combination of ex-vivo and in-vivo approaches allows a distinction between PK across skin barrier and blood clearance from skin.

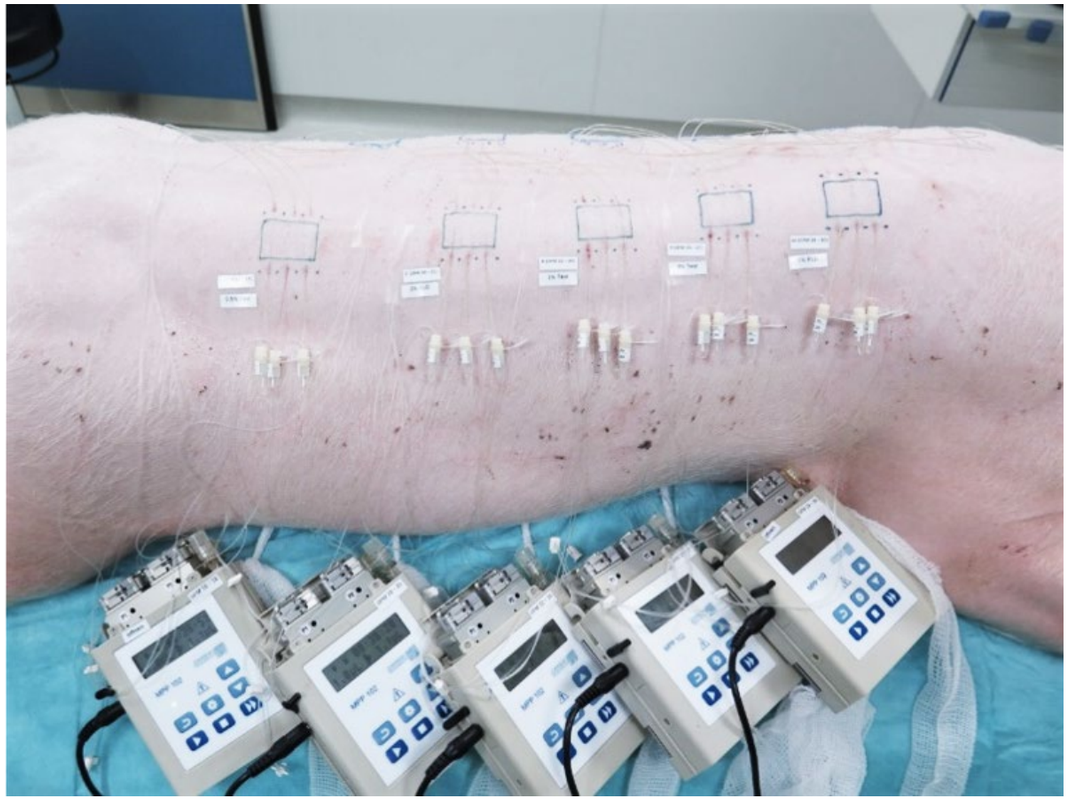
In-Vivo Preclinical Models

Lead Drug Selection Screening

- Head-to-head comparison of topical drugs
- Dermal PK drug profile
- Dermal PD profile
- Drug concentration in different areas of the skin (biopsies)
- Protein binding of substances in the skin

Benefits

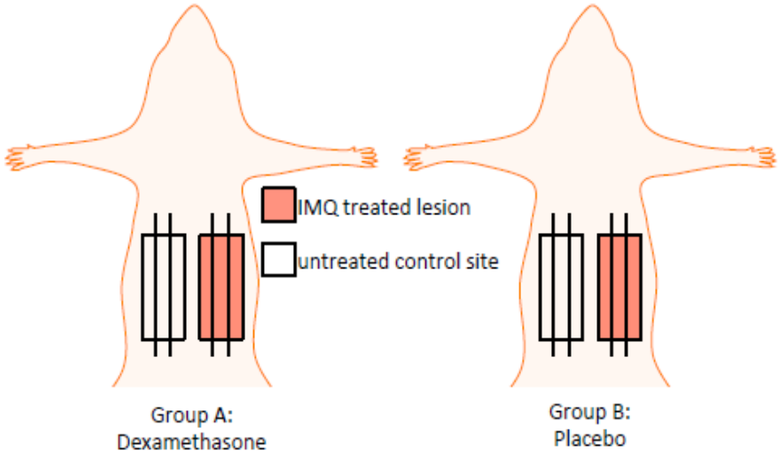
- Time-resolved monitoring
- Dermal PD profile
- Depth-resolved monitoring (biopsies)
- Early lead compound selection



Source: JOANNEUM RESEARCH

Preclinical Case Study

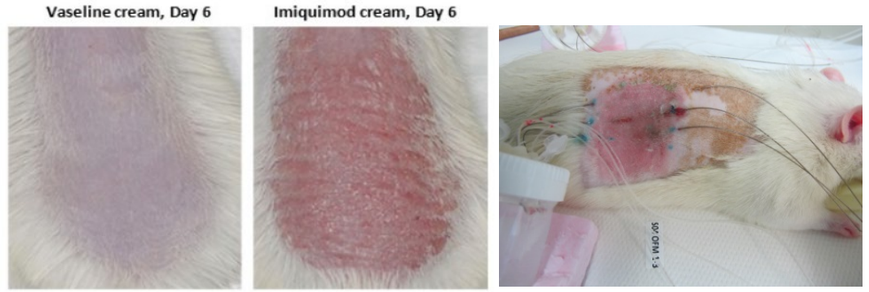
Model for proof of mechanism in psoriasis



Rats were treated once daily with imiquimod cream on a 2 x 2 cm treatment site (topical dose of 30 mg cream/cm^2).

Readout

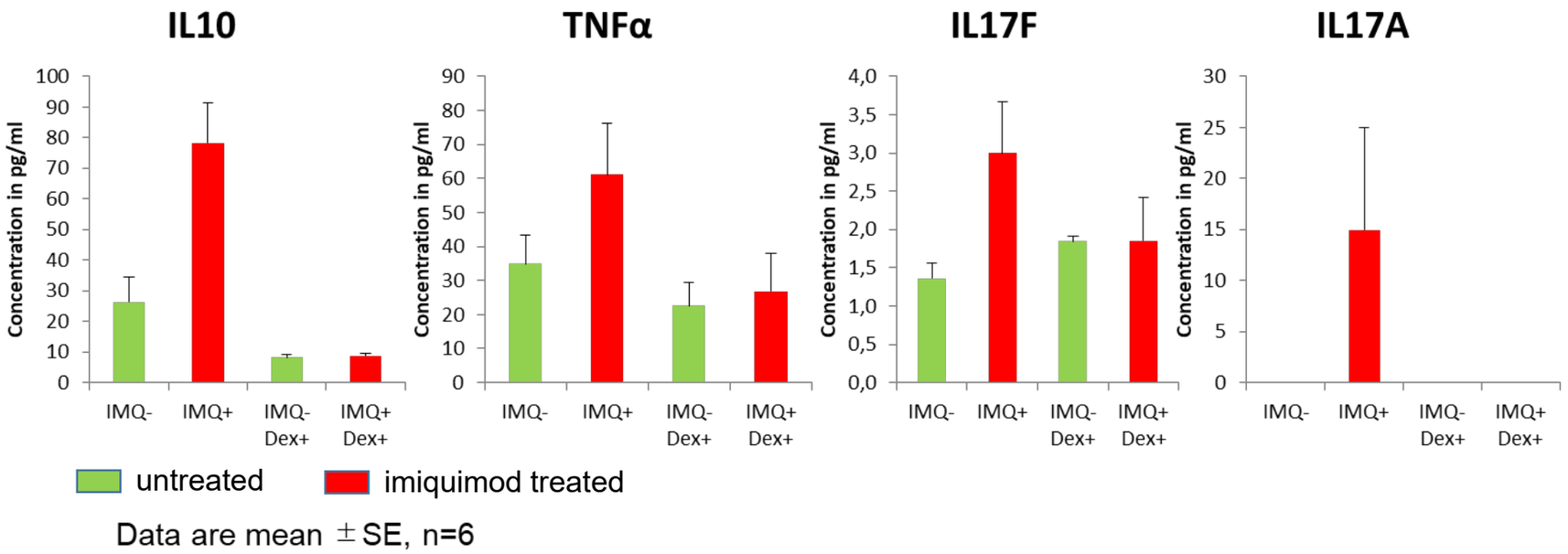
- Dermal API PK for dose-response (HPLC-MS/MS)
- Dermal PD on cytokine level (Olink)
- Dermal PD on immune cell level (FACS)
- Scoring
- Histology (biopsy)



Sources: JOANNEUM RESEARCH

Preclinical Case Study

Biomarker monitoring in psoriasis rat model

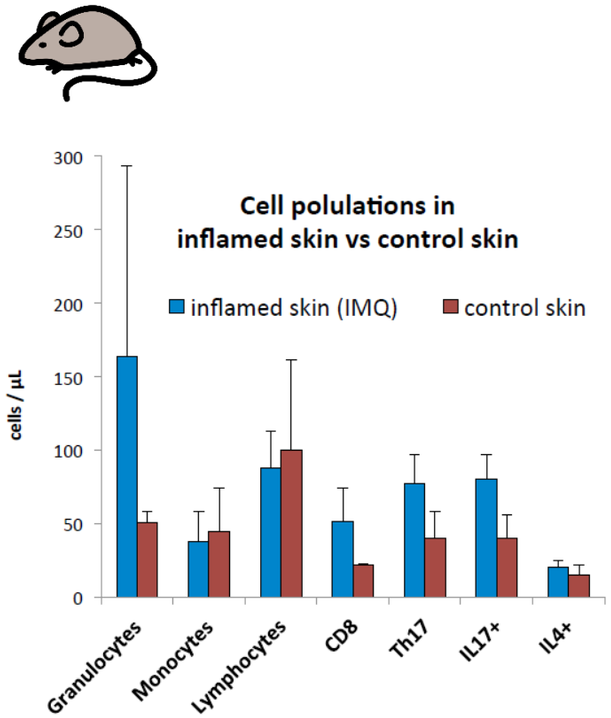


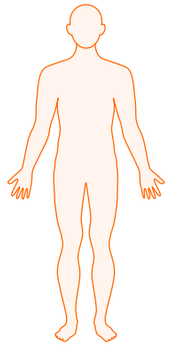
Biomarker monitoring with dOFM showed clearly different cytokine patterns in psoriatic lesion relative to control site on the same animal and demonstrated the effect of dexamethasone treatment.

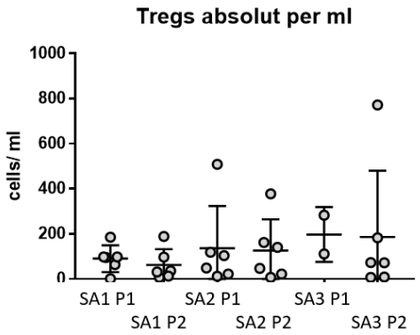
Source: JOANNEUM RESEARCH

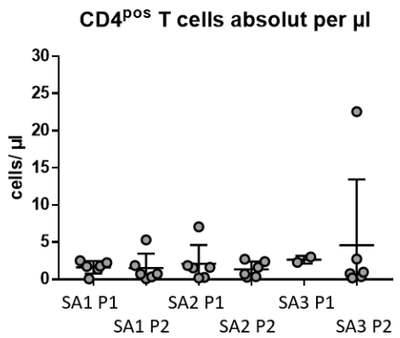
Immune cell characterization in dermal tissue

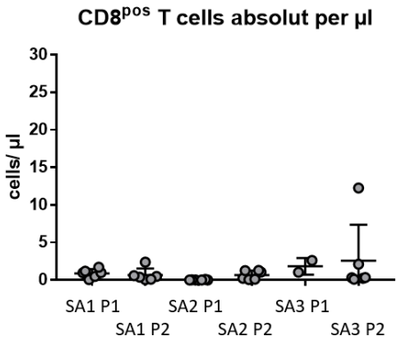
dOFM cell sampling in healthy humans







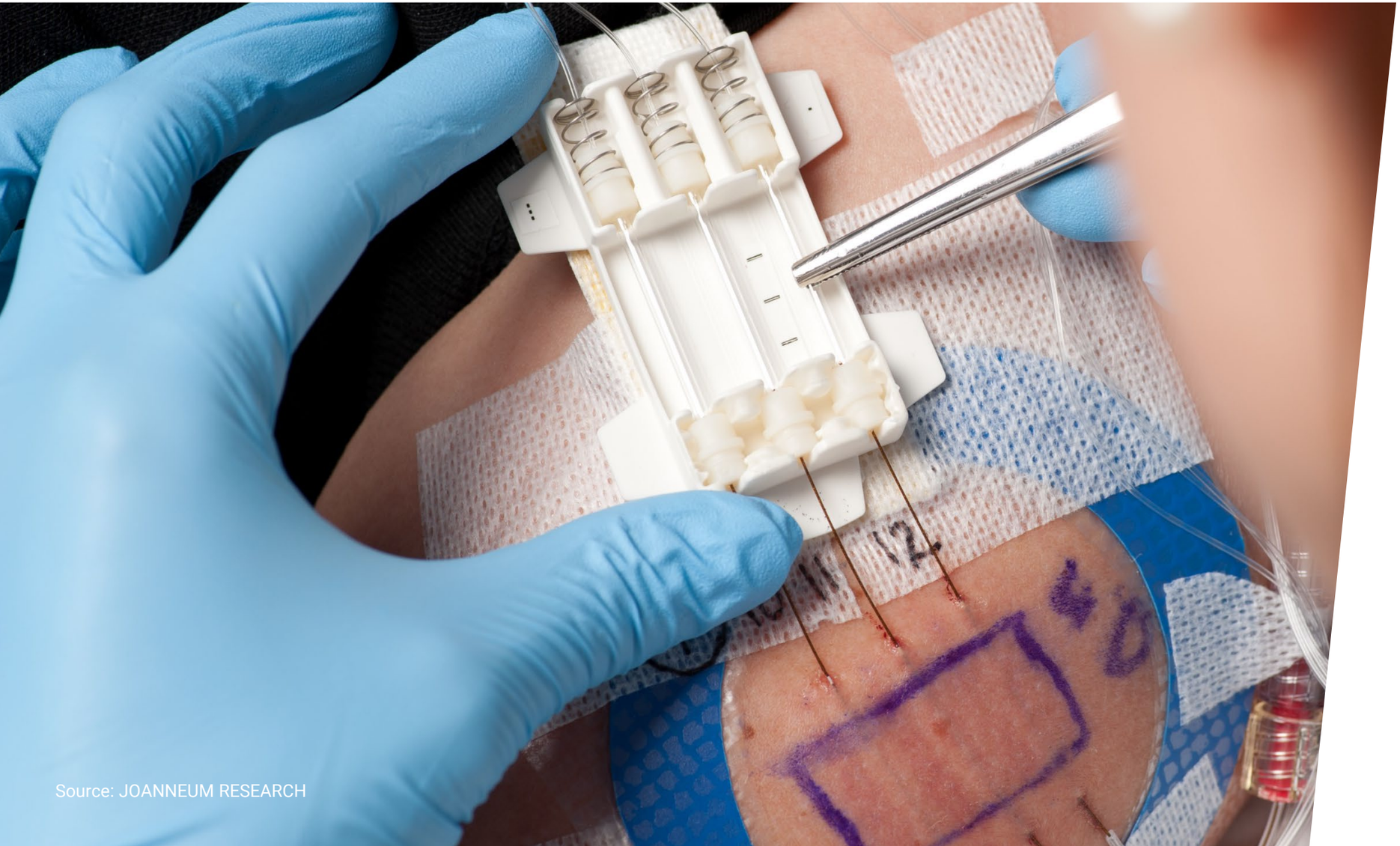




Healthy humans with three sampling sites (SA1-3), unpublished data.

Biomarker and immune cells in dermal tissue of patients with atopic dermatitis.

Characterization of the Psoriasis-like Inflammation in the Imiquimod Rat Model using Dermal Open Flow Microperfusion. Bodenlenz M, et al. 3rd Inflammatory Skin Disease Summit. 2018



Source: JOANNEUM RESEARCH

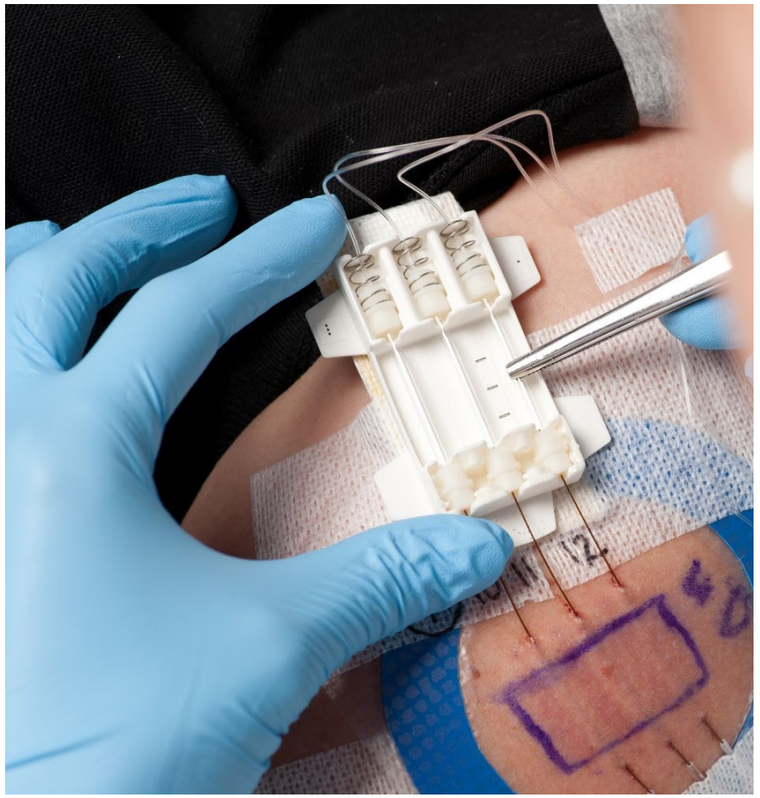
Clinical Setup & Case Studies

Clinical Setups

PK and PD studies for research and approval studies

- **Available models:** healthy and patients
- **Duration time:** up to 48 hours
- **Application sites**: up to 14 sites with 3 dOFM probes each
- **Time resolution:** 5 min to hours (dependent on sample volume for analytics)
- **OFM material:** CE certified

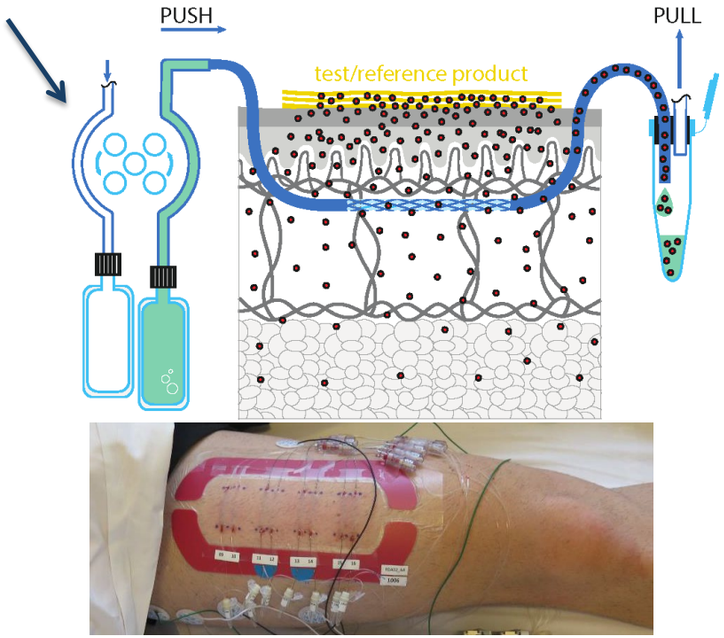
Highly standardized setup reduces inter-subject and intra-subject variability.



Clinical Setups

dOFM microdosing - minimal systemic exposure

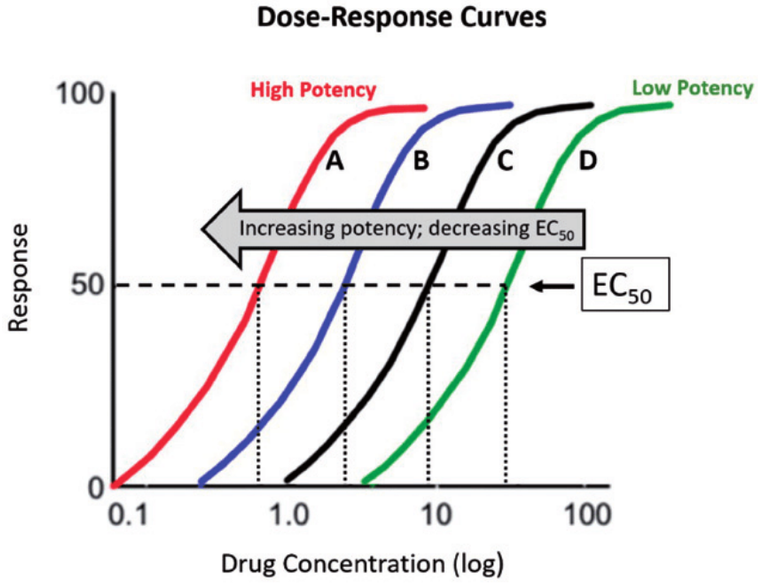
Drug candidate is added to perfusate in different concentrations.



Sources: JOANNEUM RESEARCH

Biomarker readout

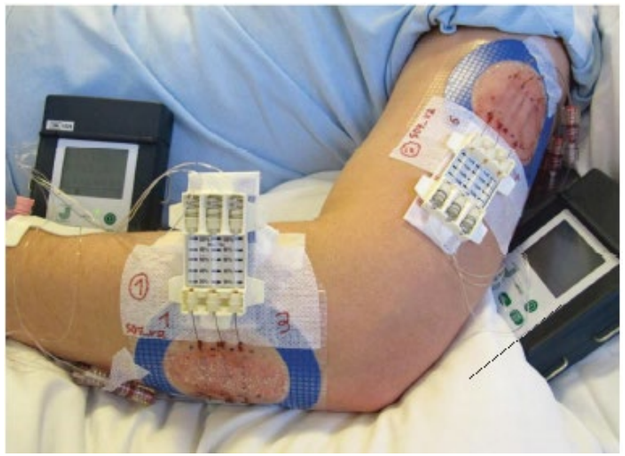
Clinical studies for proof of mechanism + dose response



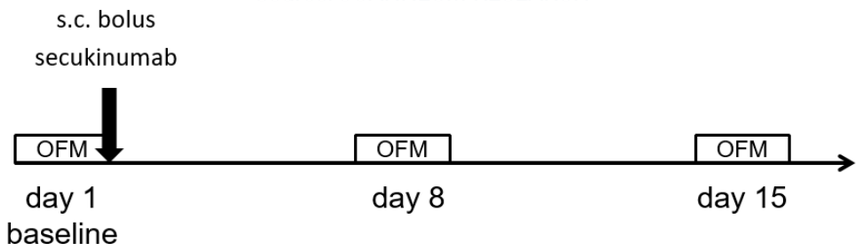
Source: 2019 Journal of Dermatological Treatment
DOI: 10.1080/09546634.2019.1643588

Clinical Case Study

PK/PD investigation of therapeutic antibody secukinumab



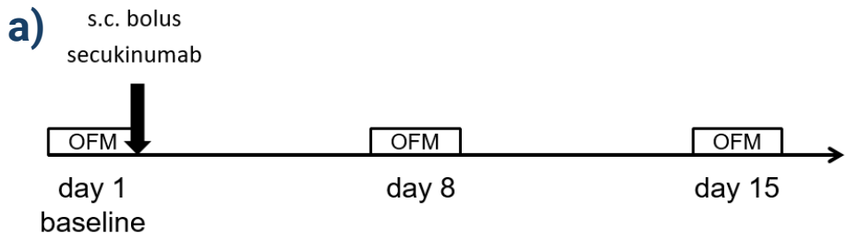
Source: JOANNEUM RESEARCH

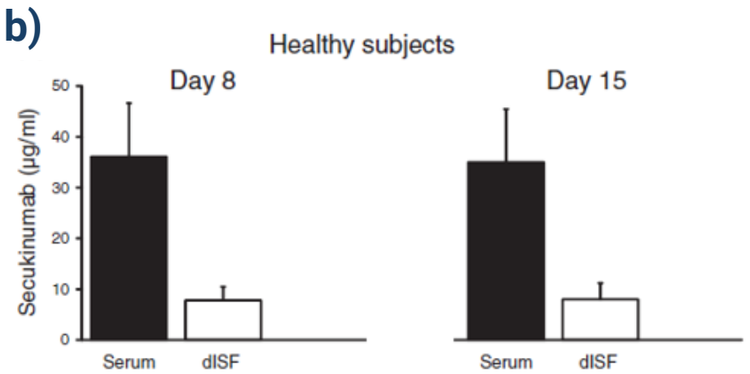


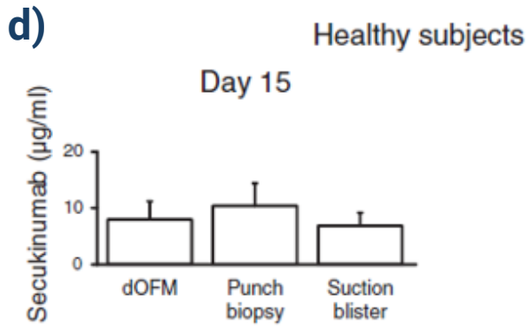
- Secukinumab: antibody drug for psoriasis (targets IL-17A)
- Clinical study in healthy volunteers and psoriasis patients
- Verification of therapeutic target (IL-17A)
- PK in blood, healthy skin, lesional skin
- Monitoring of therapeutic effect based on biomarkers
- Screening for biomarkers

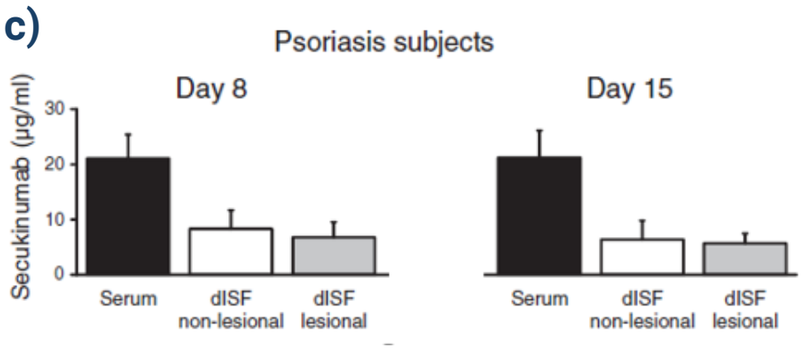
Clinical Case Study

dOFM PK data show sufficient secukinumab concentrations







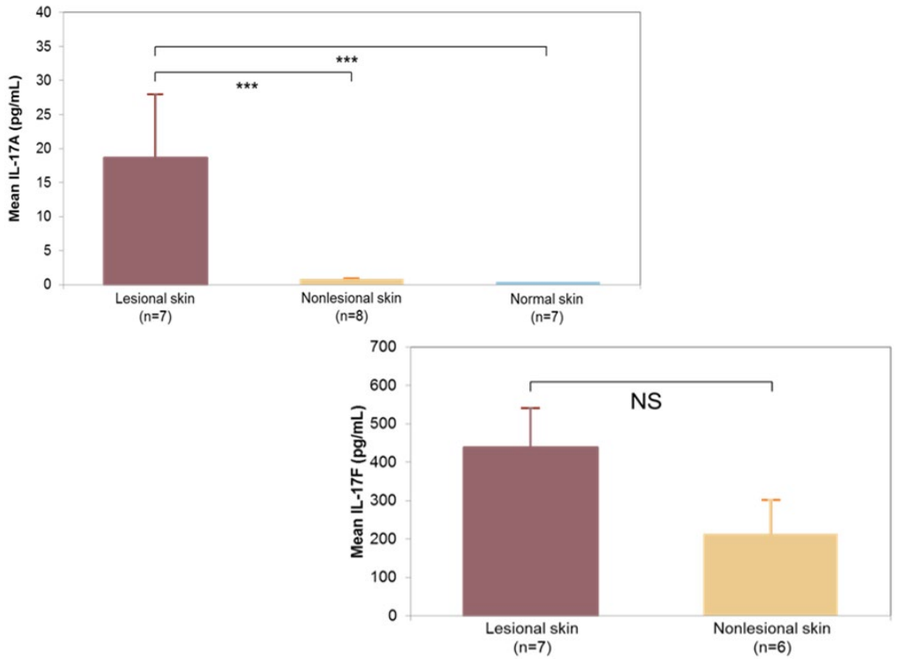


"Secukinumab distributes into dermal interstitial fluid of psoriasis patients as demonstrated by open flow microperfusion" Dragatin et al. Exp. Dermatology. 2016 doi: 10.1007/s40262-016-0442-z.– OPEN ACCESS

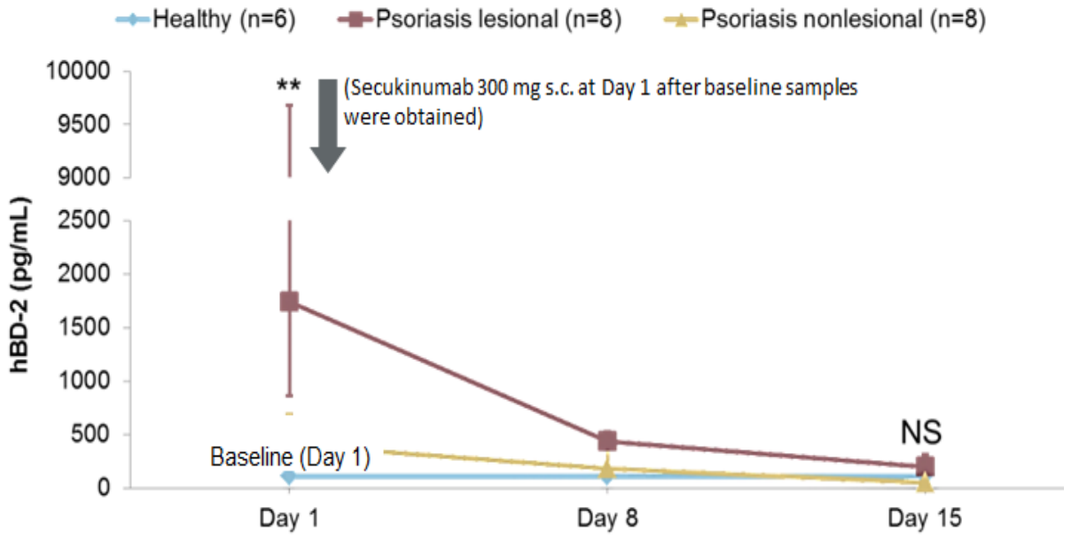
Clinical Case Study

Proof of target engagement

Verification of therapeutic target (IL-17A)



Monitoring of therapeutic effect based on downstream biomarker β-defensin

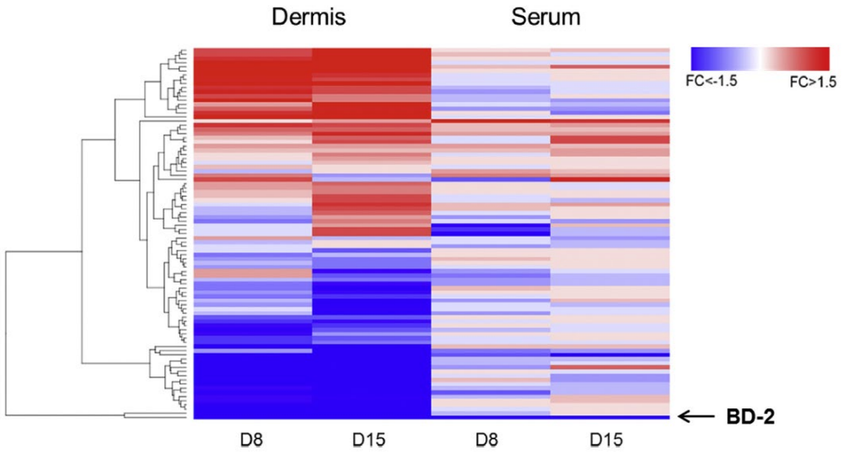


"Secukinumab distributes into dermal interstitial fluid of psoriasis patients as demonstrated by open flow microperfusion" Dragatin et al. Exp. Dermatology. 2016 doi: 10.1007/s40262-016-0442-z.– OPEN ACCESS

Clinical Case Study

dOFM increases sensitivity for biomarker screening

Different signaling pathways in the dermis of patients with psoriasis.



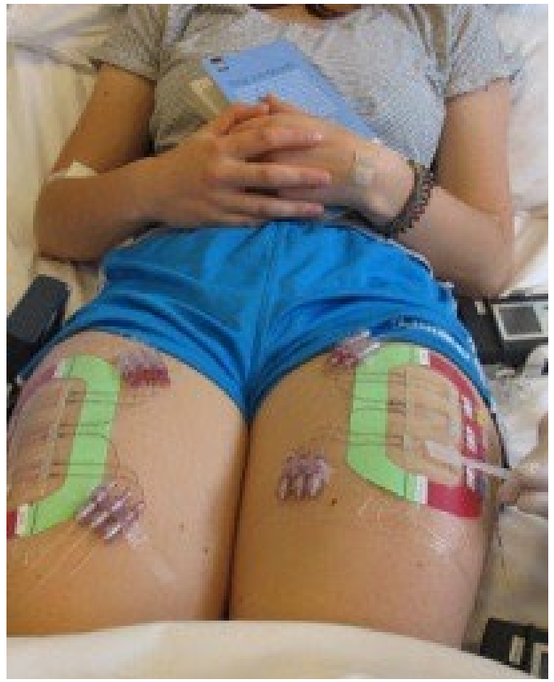
	Fold change relative to baseline			
	Dermis (dISF)		Serum	
Protein	Day 8	Day 15	Day 8	Day 15
Top 10 downregulated				
BD-2	−18.73	−32.20	−3.95	−3.66
MMP-1	−6.20	−15.19	−1.11	1.04
IL-1β	−2.71	−5.47	1.14	1.14
IL-1 receptor antagonist (IL-1ra)	−2.19	−4.37	−1.47	−2.32
MMP-8	−1.91	−3.42	−1.16	−1.07
Myeloperoxidase	−1.18	−3.20	−1.27	−1.18
CXCL1 (GRO-α, *CXCL1*)	−2.63	−3.13	−1.08	−1.17
Lipocalin-2 (NGAL, *LCN2*)	−2.14	−2.98	−1.11	−1.12
CCL20 (Macrophage inflammatory protein 3α, *CCL20*)	−2.62	−2.64	−1.24	1.45
CXCL5 (ENA-78, *CXCL5*)	−3.00	−2.50	1.05	−1.02
Other proteins of interest				
CXCL3 (GRO-γ, *CXCL3*)	−1.61	−2.20	−1.16	−1.08
CCL1 (I-309, *CCL1*)	−1.34	−1.88	1.09	1.03
TNF-α	1.00	1.18	1.04	1.03
Top 5 upregulated				
Endoglin	2.51	2.52	1.04	1.08
Leptin	2.59	2.62	1.09	1.39
Adiponectin (Acrp-30)	1.50	2.72	1.13	−1.04
Eotaxin-2 (*CCL24*)	1.56	2.77	1.06	1.14
IgE	1.92	3.19	−1.00	−1.06

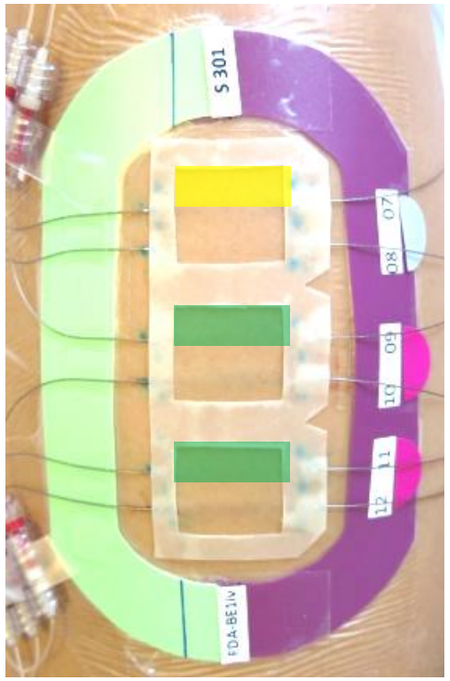
"β-Defensin 2 is a responsive biomarker of IL-17A-driven skin pathology in patients with psoriasis" Kolbinger et al. J Allergy Clin Immonol. 2016 doi: 10.1016/j.jaci.2016.06.038

Bioequivalence Clinical Case Study

dOFM use for PK-based BE for acyclovir

Measurement of topical dermal drug BE on PK level





Clinical study outline - acyclovir:

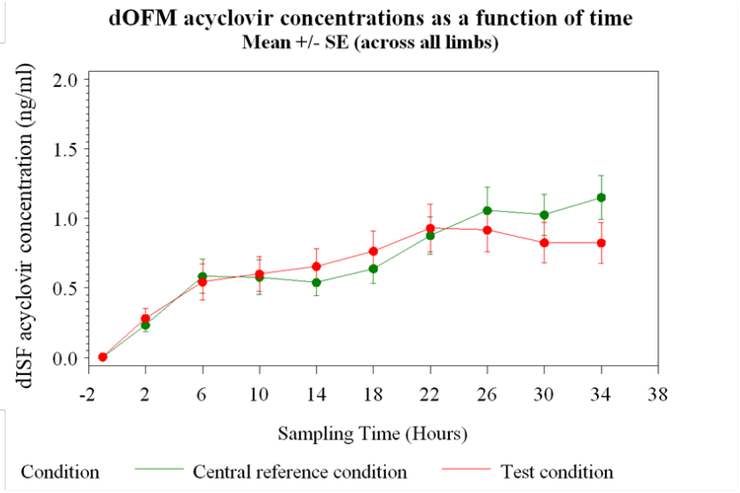
- 20 healthy subjects
- Reference Listed Drug (R): Zovirax® US
- Test Product (T): Aciclovir-1A Pharma Austria
- 36 hours dOFM sampling time

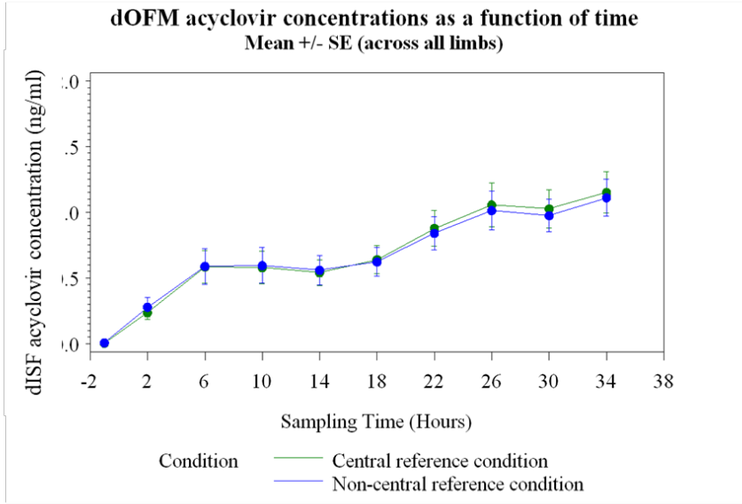
Funding for this project was made possible, in part, by the Food and Drug Administration through grants 1U01FD004946 and 1U01FD005861. The views expressed in this presentation do not reflect the official policies of the Food and Drug Administration, or the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government. The human research study was approved by the FDA Research Involving Human Subject Committee (RIHSC) and the local Institutional Review Board (IRB) of the Medical University Graz, Austria

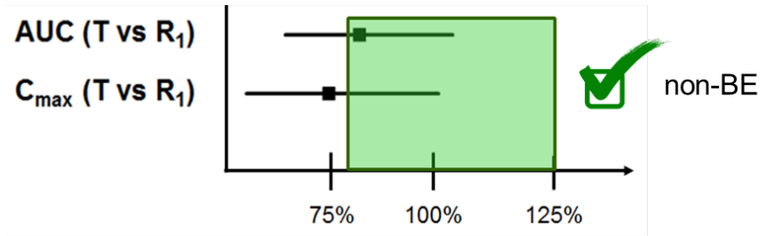
Bioequivalence Clinical Case Study

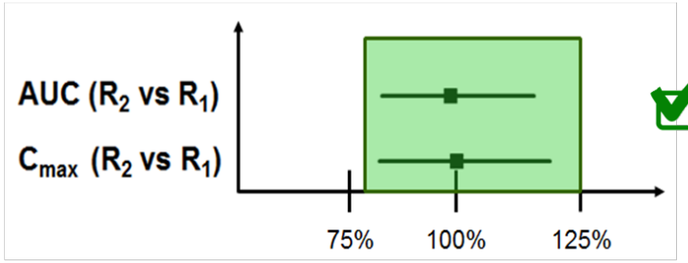
dOFM shows its ability to investigate bioequivalence: acyclovir

dOFM allows measurement of topical dermal drug BE on PK level.





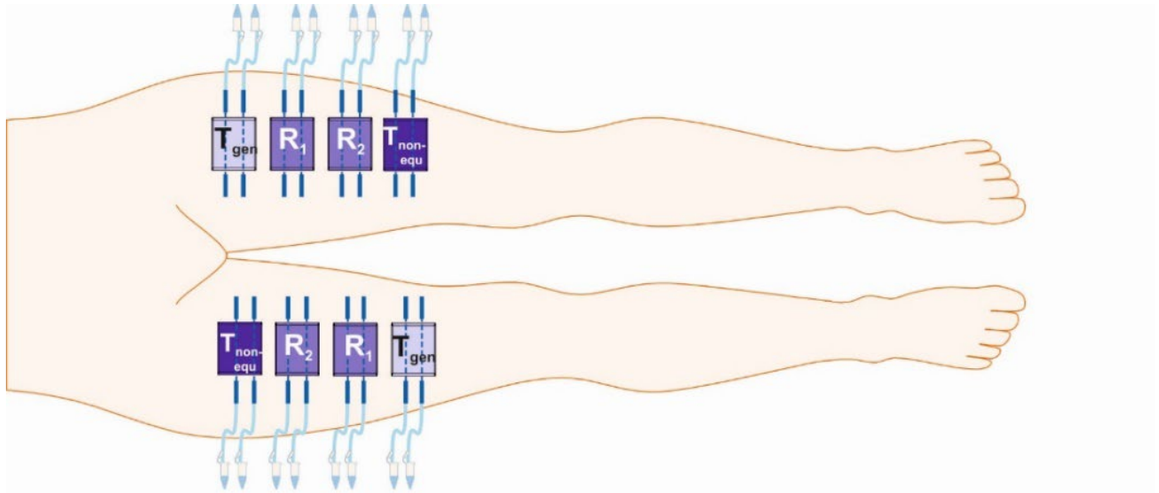




"Open Flow Microperfusion as a Dermal Pharmacokinetic Approach to Evaluate Topical Bioequivalence" Bodenlenz et al. Clin. Pharmacokinet. 2017 doi: 10.1007/s40262-016-0442-z.– OPEN ACCESS

Bioequivalence Clinical Case Study

dOFM use for PK-based BE for lidocaine and prilocaine



R_1/R_2 15mg/cm² EMLA® topical cream (Actavis Pharma Inc., US)
$T_{non\text{-}equ}$ 15 mg/cm² Oraqix periodontal gel (Dentsply Detrey GmbH, Germany)
T_{gen} 15 mg/cm² Lidocaine 2.5% and Prilocaine 2.5% cream (E. Fougera & Co, US)

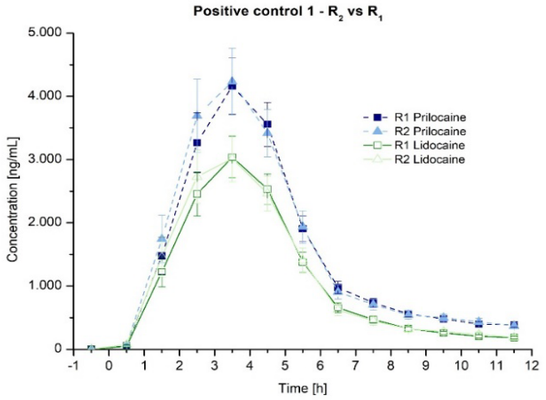
The U.S. Food and Drug Administration recently published a video showing the potential of dermal open flow microperfusion (dOFM) for generic drug development. Watch it!

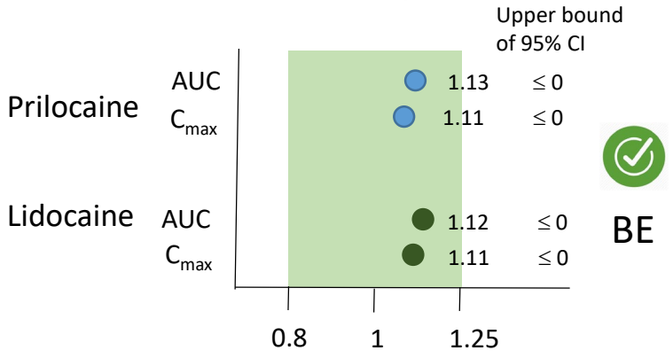
Watch the 2020 presentation on dOFM for topical generic bioequivalence at "Advancing Innovative Science in Generic Drug Development Workshop September 29 & 30, 2020". Watch it!

Bioequivalence Clinical Case Study

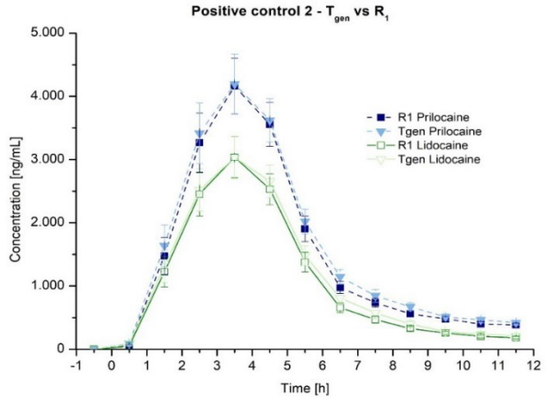
dOFM shows its ability to investigate bioequivalence: lidocaine + prilocaine

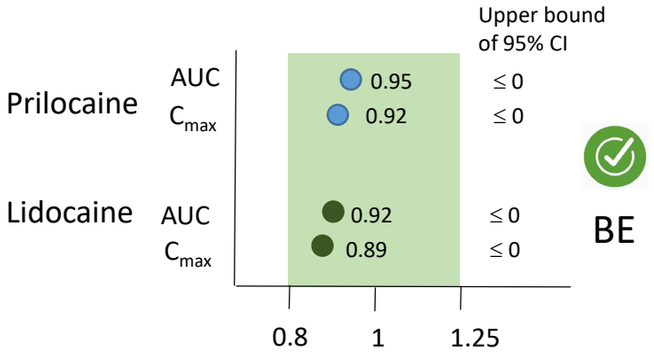
EMLA vs. itself (RLD)



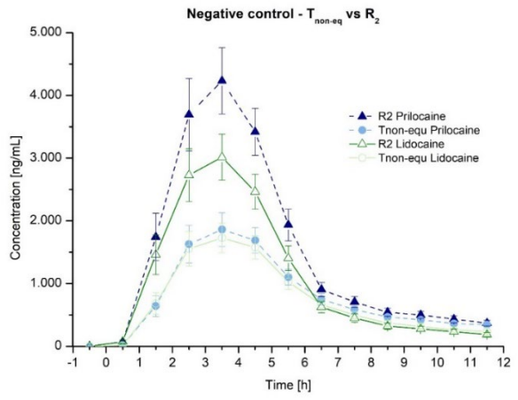


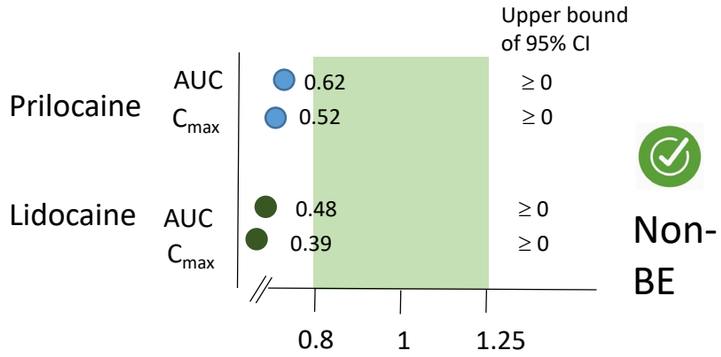
US-FDA approved Generic vs. RLD (EMLA)





Non-BE drug vs. EMLA





"A Clinical Study to Assess the Bioequivalence of Lidocaine and Prilocaine Topical Drug Products Using Dermal Open Flow Microperfusion" Tiffner et al. 2020. Poster @ AAPS PharmaSci3060 Meeting

Key Learnings

dOFM improves your dermal drug development by

... providing direct unfiltered access to dermal tissue.

... reducing risk, costs, and time, using high quality dermal PK and PD data.

dOFM monitors local drug concentration in the dermis and

... allows PK and PD investigation in the target tissue.

... shows higher sensitivity and lower variability compared to state-of-the-art dermal methods.

dOFM allows a head-to-head comparison in the same subject and enables dermal bioequivalence determination in a low number of healthy subjects.

dOFM enables a link across ex-vivo, pre-clinical and clinical results by using identical dOFM setups.

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Thank you for your attention

dOFM