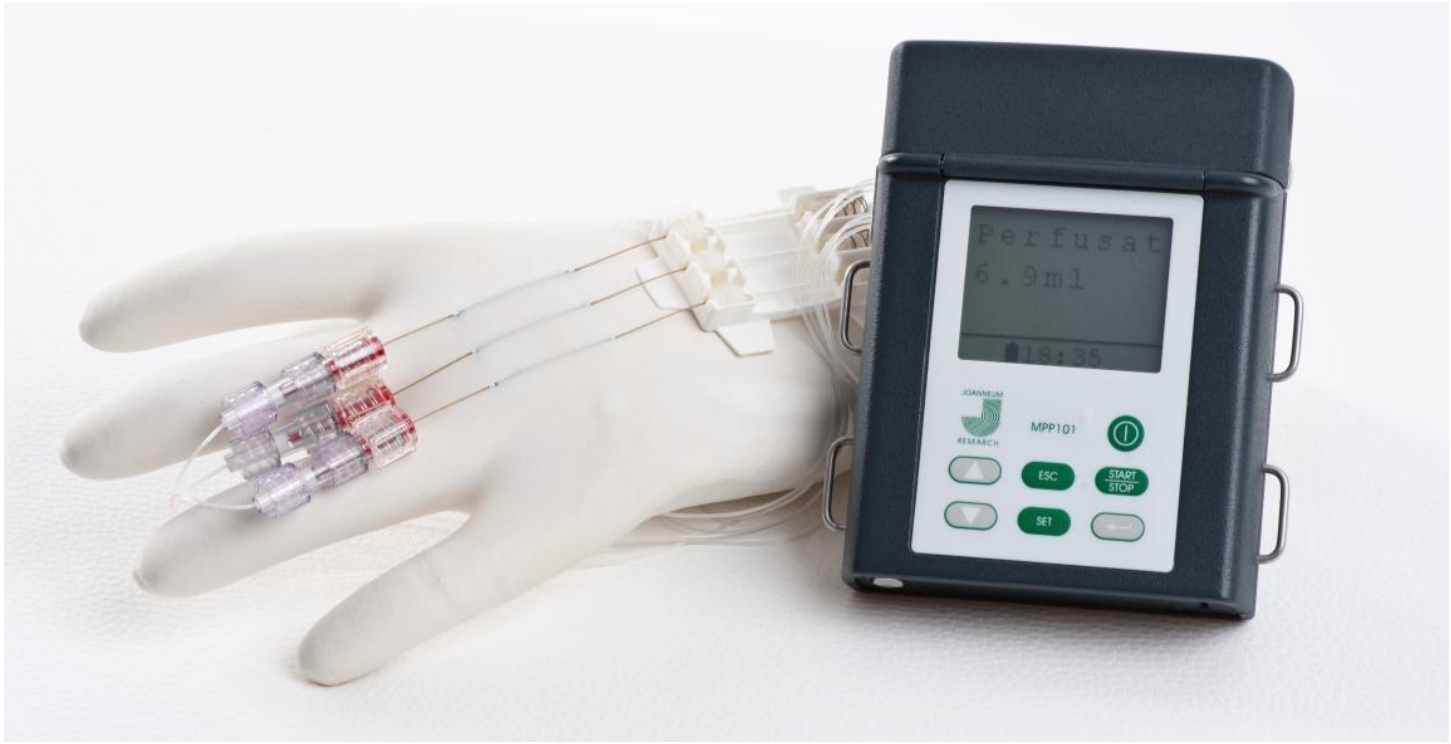
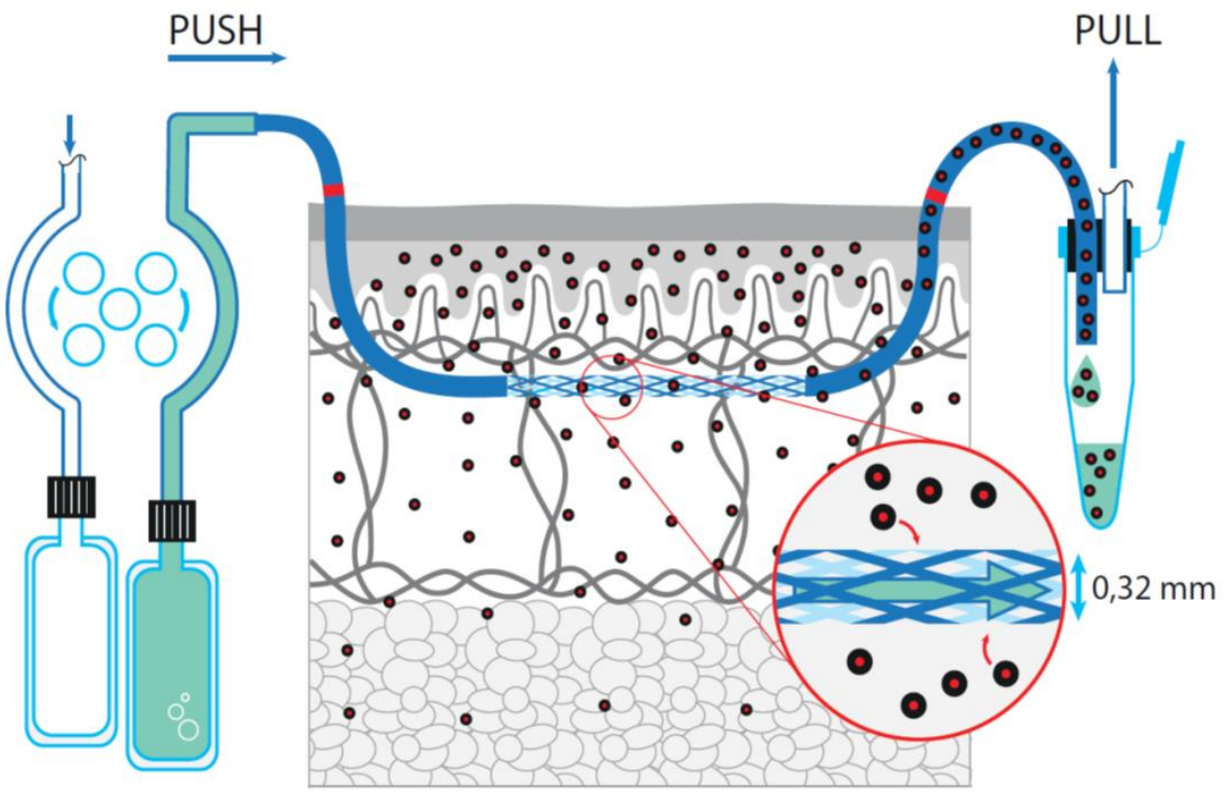
Innovative dermal PK and PD

In-vivo proof of mechanism for clinical dermal bioequivalence using OFM

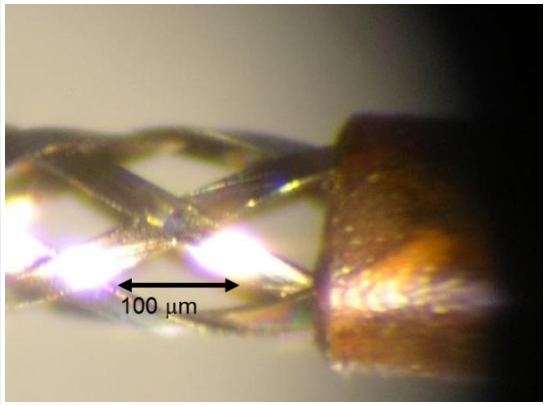


Content

Open Flow Microperfusion



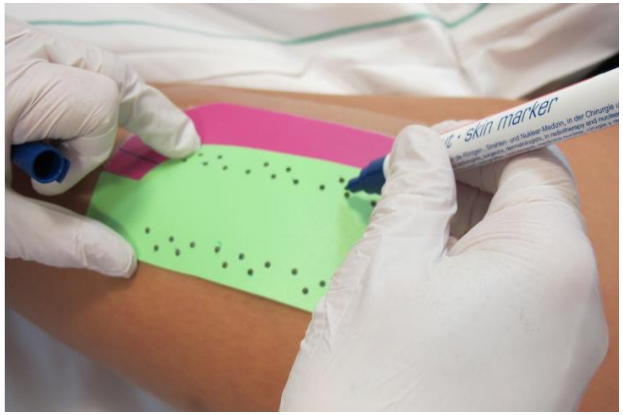
CE certified for clinical use

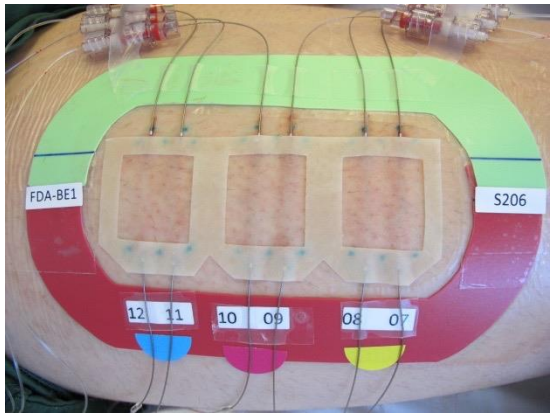


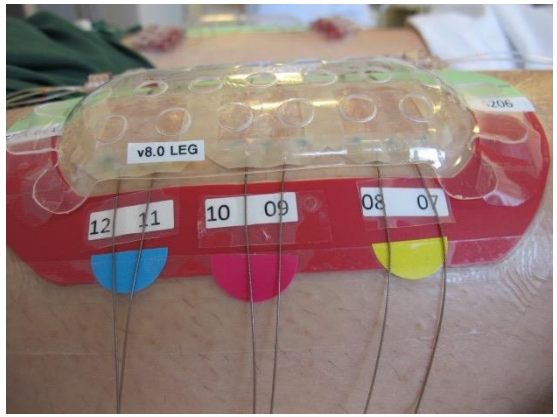
OFM samples represent diluted but unfiltered interstitial fluid

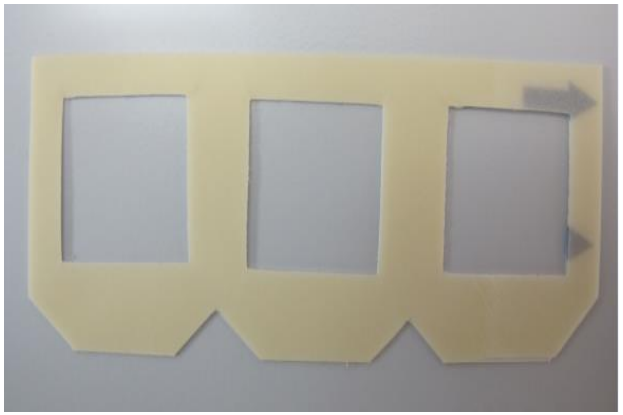
dOFM

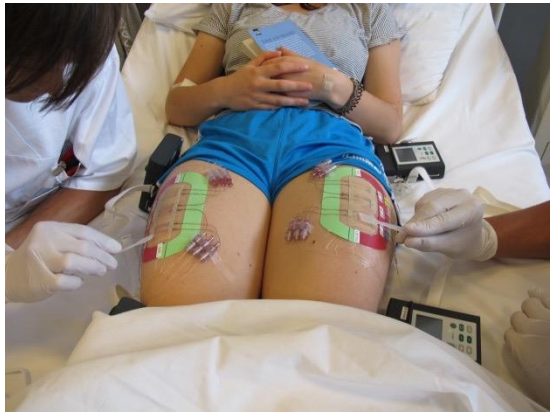
set-up

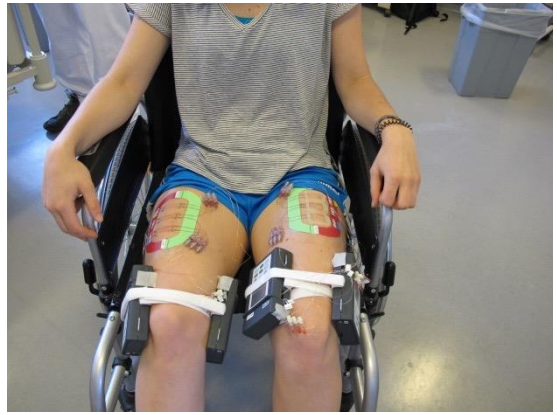












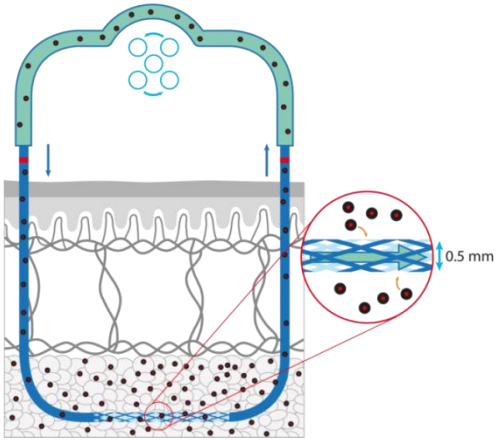
All procedures are highly standardized

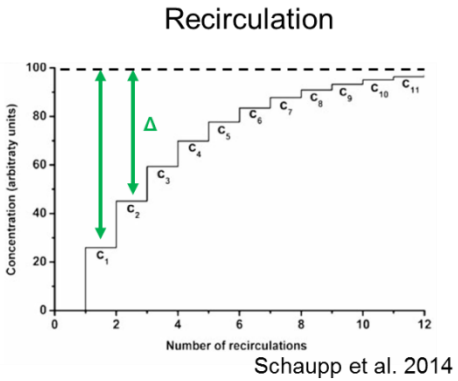
Content

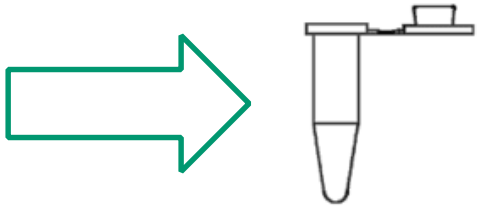
„pure“ Interstitial Fluid

Investigate your API in a realistic matrix

OFM recirculation is used to achieve an equilibrium between perfusate and interstitium.







“pure” ISF

add API — dialysis

stability investigation ⟵ ⟶ **Free fraction**

add API

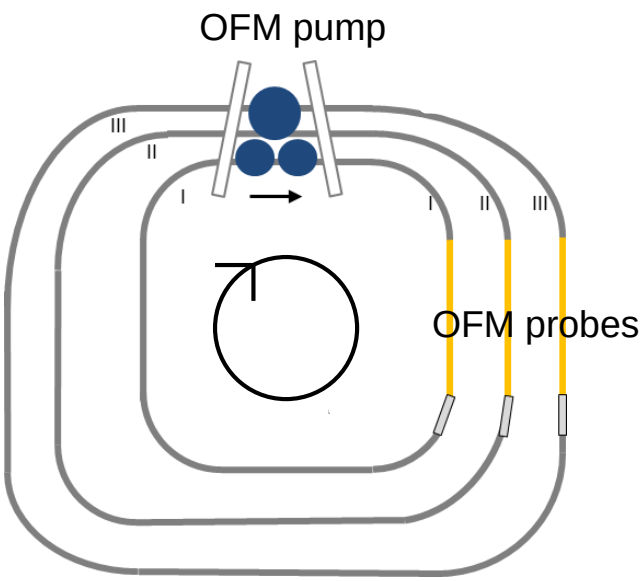
metabolism investigation ⟵

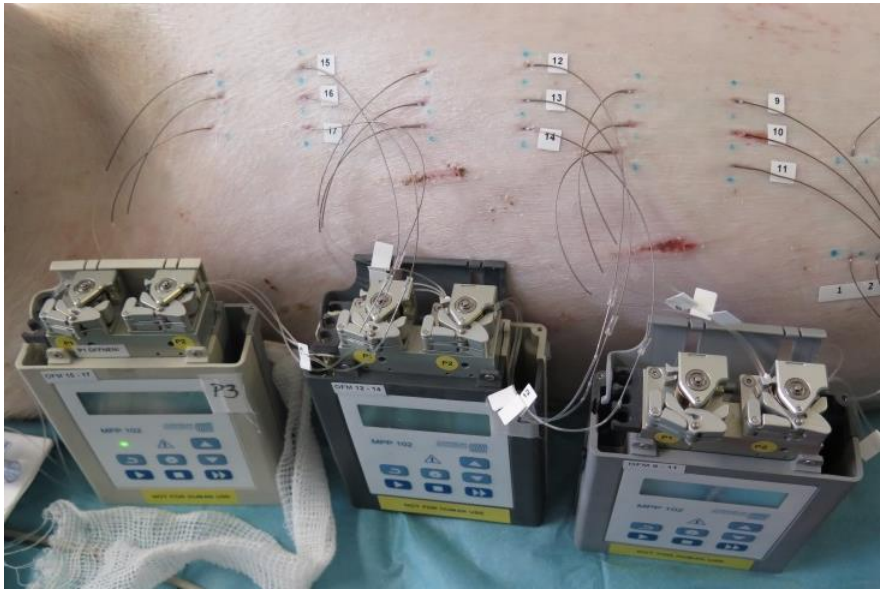
API stability and degradation used for API design and toxicity

„pure“ Interstitial Fluid

Investigate your API in a realistic matrix

Recirculation of physiological saline in the anesthetized enabled sampling of „pure“ interstitial fluid (ISF) in pigs



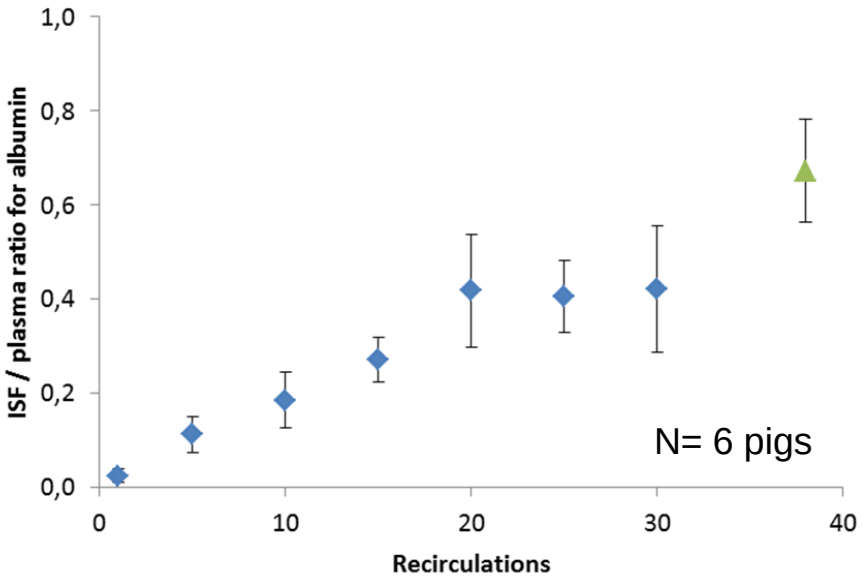


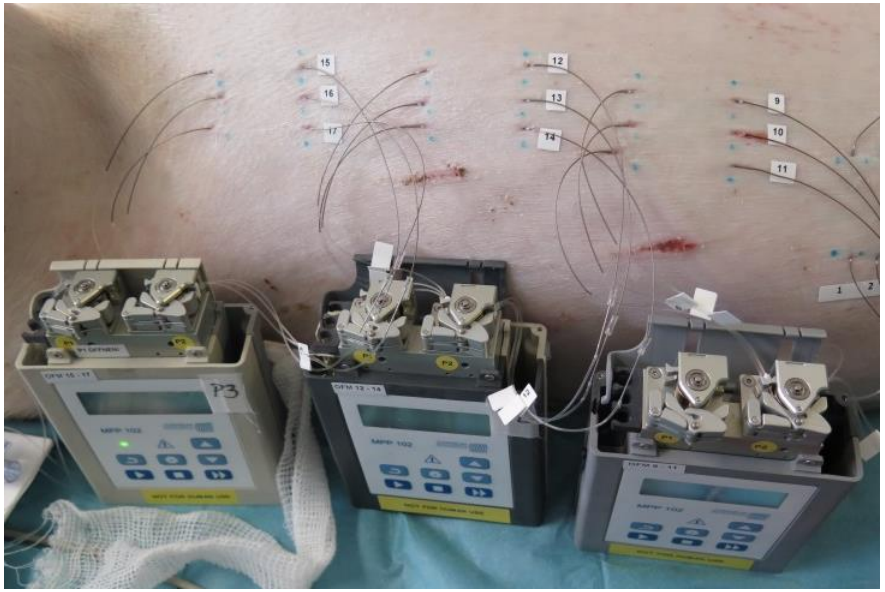
„pure“ Interstitial Fluid

Investigate your API in a realistic matrix

- 20 recirculations are sufficient to achieve plateau phase for albumin
- Lymph showed higher albumin concentration than „pure“ ISF

  ➔ Lymph represents a different compartement than interstitium





Recirculation-OFM is able to sample “pure” ISF

Content

Prediction of Drug Effect

skin penetration and dermal metabolism

Case Study (Leo Pharma)[1]

AIM: Development of a topical drug for AD treatment which has

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

➔ **PDE4 inhibitors with high in vivo clearance and adequate skin stability**

API candidates:

- A: low Mw, LogD ~3, human unbound fraction ~2%, *in vitro* skin model: stable, EC50* ~80nM, ….
- B: low Mw, LogD ~3, human unbound fraction ~5%, *in vitro* skin model: stable, EC50* ~60nM, …

➔ **Both compounds show in-vitro activity and were selected for clinical development**

*EC50 is based on in vitro inhibition of LPS induced TNFalpha release from human PBMCs

[1] unpublished results: from Leo Pharma: Maja Lambert, Stefan Eirefeldt, Fredrik Johansson, Line Hollesen Basse, Malene Bertelsen, Jens Larsen, Simon Feldbæk Nielsen

Prediction of Drug Effect

skin penetration and dermal metabolism

Case Study (Leo Pharma)[1]

Ex-Vivo Human Skin Punch Biopsies

- **A**: [API] > factor 10 higher than EC50
- **B**: [API] > factor 100 higher than EC50

➔ **Both compounds are good candidates for clinical evaluation**

Clinical Trial

- **A** demonstrated clinical efficacy in AD patients (phase 2) in a 4 wk proof of concept study with twice daily dermal application of a cream formulation in different strengths of the cream vehicle and Elidel cream. Biopsy concentrations were determined at 10 µM.
- **B** showed now difference to cream vehicle in a clinical study with AD patients 3 wk with twice daily dermal application of cream formulation compared to cream vehicle. Biopsy concentrations were determined at 6 µM.

➔ **Punch biopsies revealed API concentration well over EC50 but B showed no treatment effect.**

[1] unpublished results: from Leo Pharma: Maja Lambert, Stefan Eirefeldt, Fredrik Johansson, Line Hollesen Basse, Malene Bertelsen, Jens Larsen, Simon Feldbæk Nielsen

Prediction of Drug Effect

skin penetration and dermal metabolism

Case Study (Leo Pharma)[1]

Ex-Vivo Fresh Human Skin OFM Model

- Determination of 24 hour dermal concentration profile for API

➔ **Elimination of punch biopsy contamination due to remaining drug at SC**

➔ **Focus on the relevant compartment → DERMIS to reflect effective API concentration**

RESULTS

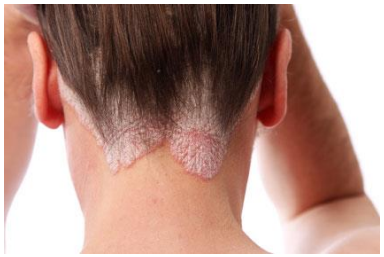
- A:[API] more than 10 fold lower compared to biopsies but higher than EC50
- B: [API] more than 10 fold **lower** compared to biopsies and below EC50

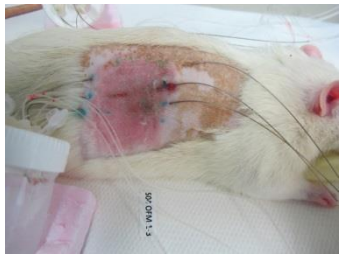
OFM allows a realistic determination of API PK profiles to predict clinical efficacy, essential in the absence of reliable biomarker

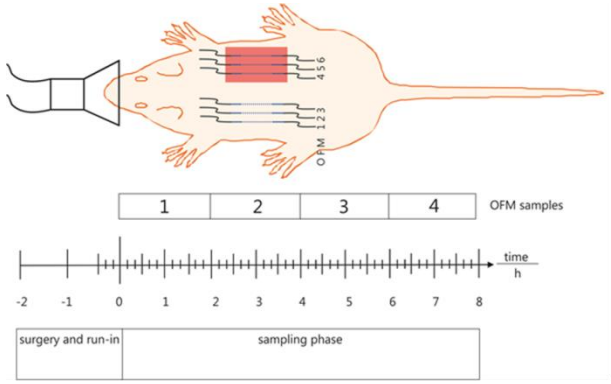
Content

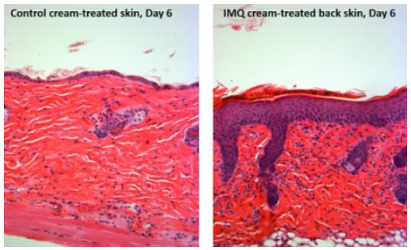
Preclinical Proof of Concept
mode of action of your API in-vivo

Case Study (undisclosed sponsor)[2]









Dermal API PK for dose-response ← HPLC-MS/MS

Dermal PD effect on cytokine level ← OLINK

Dermal PD on immune cell level ← FACS

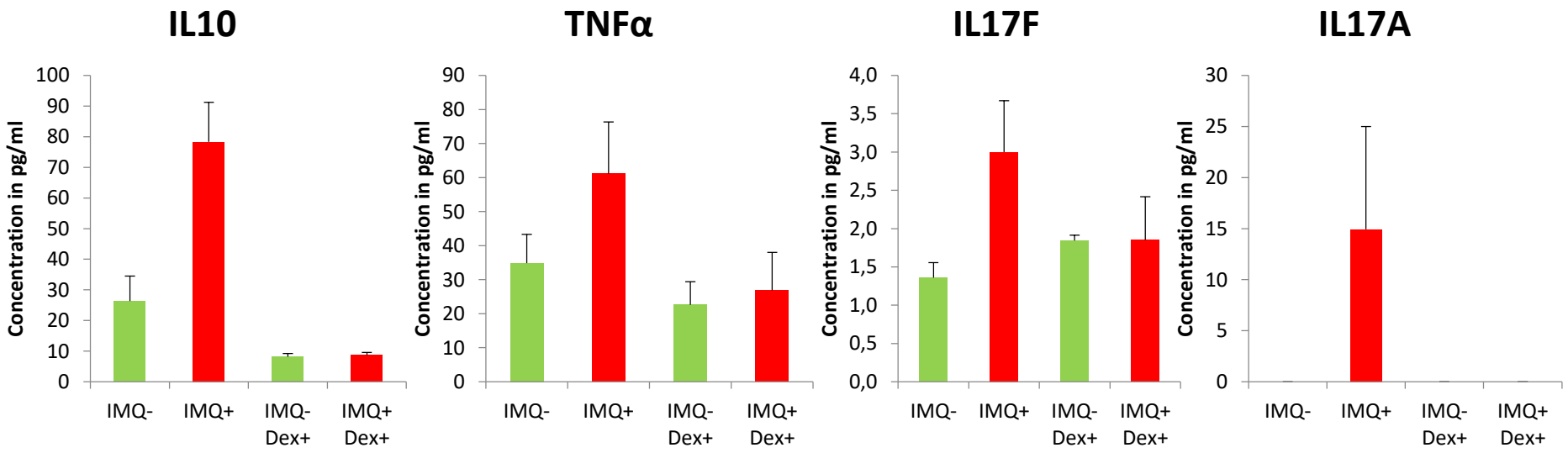
In-vivo effect of API on cytokine and immune cell level

Preclinical Proof of Concept
mode of action of your API in-vivo

Case Study (undisclosed sponsor)[2]

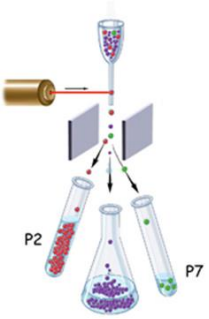
rats	Topical treatment	Inhibition treatment	Label
S01-S06	Untreated	Untreated	IMQ-
S01-S06	IMQ8d	Untreated	IMQ+
S07-S12	Untreated	Dex2mg	IMQ- Dex+
S07-S12	IMQ8d	Dex2mg	IMQ+ Dex+

[2] unpublished results



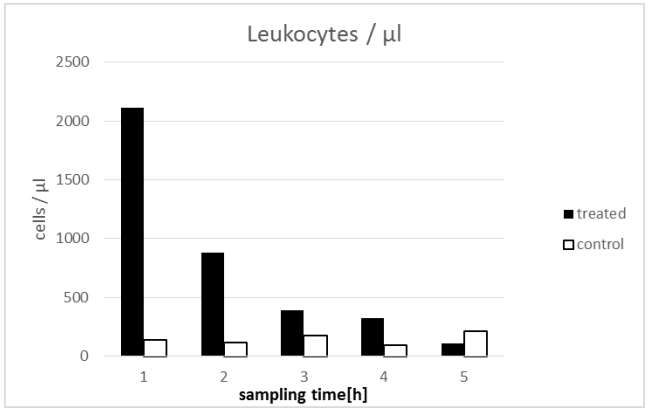
Data are mean ±SE, n=6; 8 days of treatment

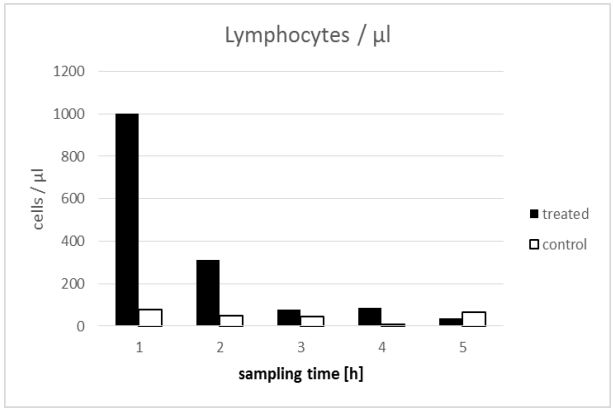
IMQ-Rat Model is an in vivo model for psoriatic inflammation



Preclinical Proof of Concept

mode of action of your API in-vivo

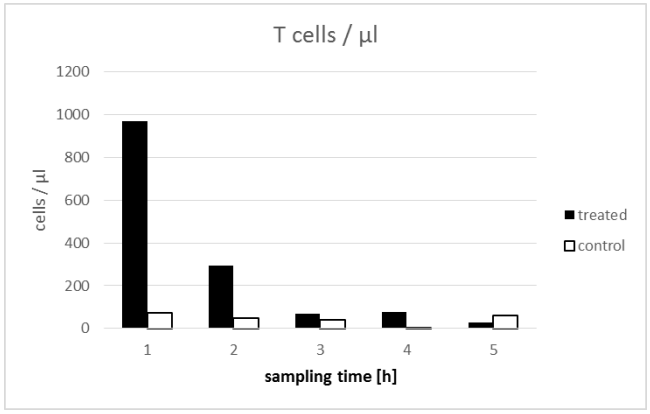


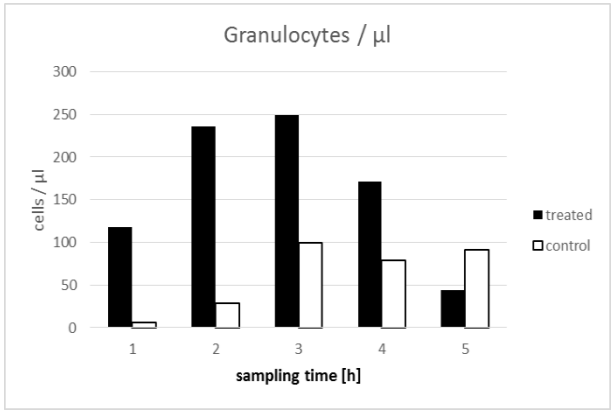


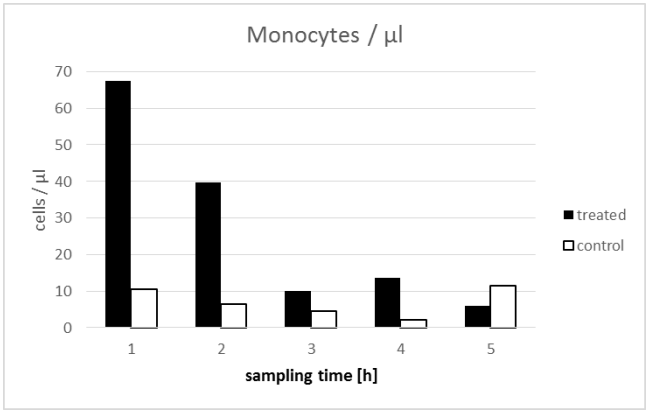
[2] unpublished results

lesional

control

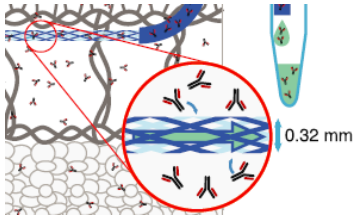






This psoriasis animal model allows for PK and PD investigations

Content

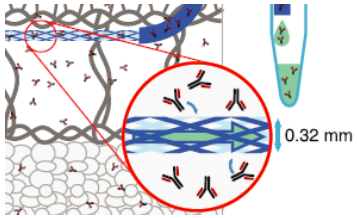


OFM

PK/PD of an Antibody Drug: Case Study Secukinumab

Background and Objectives

- Secukinumab, a fully human monoclonal antibody that selectively targets IL-17A, has demonstrated efficacy in phase 3 trials, within 16 weeks of initiation of treatment.
- The objective of this exploratory, single-center, open-label study (NCT01539213) was to further characterize the mechanism of action of secukinumab in the skin in
  - 8 healthy volunteers (Part 1)
  - 8 plaque psoriasis patients (Part 2)
- OFM was performed on Day 1, 8 and 15 in Part 1 and 2



OFM

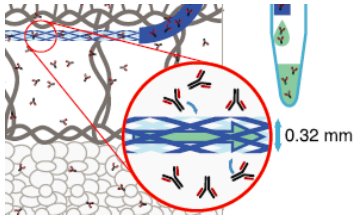
PK/PD of an Antibody-Drug: Case Study Secukinumab

Primary Aim

- Absolute quantification of Secukinumab in the dermis of healthy volunteers and psoriatic patients

Secondary Aims

- Investigate that postulated signaling pathways are different in healthy and psoriatic patients in dermis - IL17a pathway
- Investigate postulated mode of action -down stream IL17a marker
- Investigate drug effect on a protein level - mediator for keratinocyte proliferation and angiogenesis and keratinocyte mobility



JOANNEUM RESEARCH HEALTH

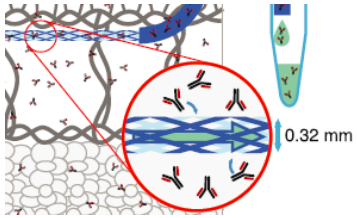
OFM

PK of an Antibody-Drug: Case Study Secukinumab

Primary Aim: Absolute quantification of secukinumab in the dermis of healthy volunteers and psoriasis patients

Serum and Dermal Secukinumab Levels (µg/mL, mean ± SD)					
Healthy Volunteers (n = 8)					
Serum		Dermal ISF[a,b]		Skin biopsy[c]	Blister fluid
Day 8	Day 15	Day 8	Day 15	Day 15	Day 15
36.1 ± 10.5	35.0 ± 10.5	7.76 ± 1.30	8.02 ± 3.23	10.40 ± 3.97	6.89 ± 2.26

Dermal ISF concentrations ~22% of serum
Dermal concentration by OFM, blister fluid, biopsies are comparable.



JOANNEUM RESEARCH HEALTH

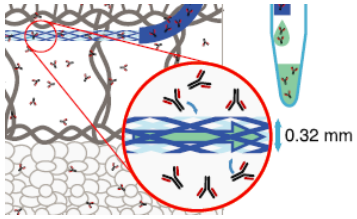
OFM

PK of an Antibody-Drug: Case Study Secukinumab

Primary Aim: Absolute quantification of Secukinumab in dermis in healthy volunteers and psoriatic patients

Serum and Dermal Secukinumab Levels (µg/mL, mean ± SD)					
Psoriatic Subjects (n = 8)					
Serum		Dermal ISF[a,b]			
Day 8	Day 15	Day 8		Day 15	
		L	NL	L	NL
21.1 ± 4.3	21.2 ± 4.9	6.76 ± 2.68	8.34 ± 3.35	5.65 ± 1.80	6.39 ± 3.35

Dermal ISF concentrations are 28-39% of serum concentration. Dermal ISF concentrations on day 8 and day 15 are similar.

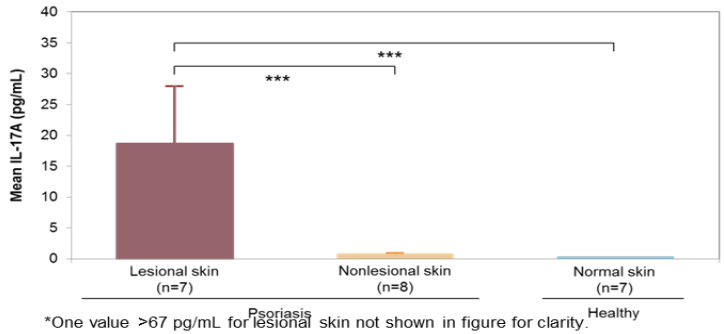


OFM

PD of an Antibody-Drug: Case Study Secukinumab

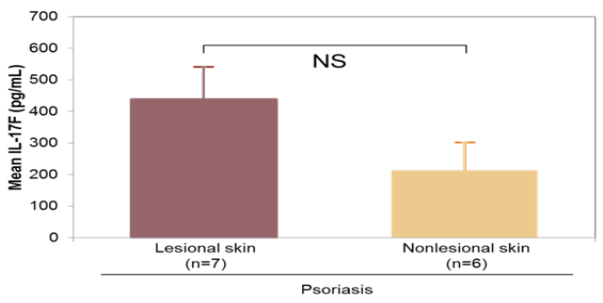
Secondary Aim: Investigate that postulated signaling pathways are different in healthy volunteers and psoriatic patients in dermis - IL17a pathway

Baseline IL-17A in Skin of Psoriasis Subjects and Healthy Volunteers (Mean + SE)



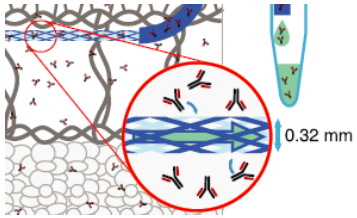
*One value >67 pg/mL for lesional skin not shown in figure for clarity.
****P* < 0.0001, lesional vs. non-lesional skin and lesional vs. healthy skin.
Calculated absolute values take into account mean relative recovery rate of the reference molecule (sinistrin).
The data below LLOQ were imputed by ½ LLOQ.

Baseline IL-17F in Skin of Psoriasis Subjects (Mean + SE)



Calculated absolute values take into account mean relative recovery rate of the reference molecule (sinistrin).
The data below LLOQ were imputed by ½ LLOQ.

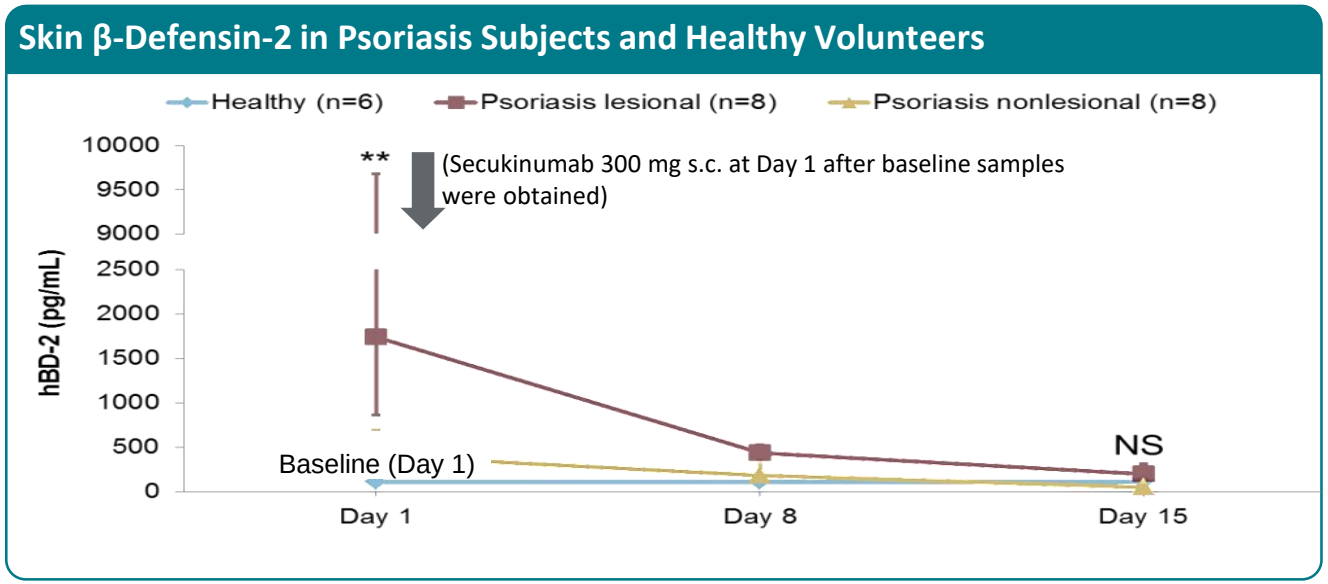
IL-17A, but not IL-17F, is significantly higher in psoriatic lesional skin compared with non-lesional skin or skin of healthy volunteers



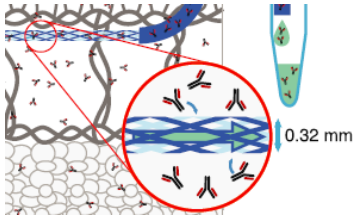
OFM

PD of an Antibody-Drug: Case Study Secukinumab

Secondary aim: Investigate postulated mode of action - downstream IL17a marker



ß-defensin-2 protein levels are elevated in psoriatic lesional skin and serum and decrease rapidly in response to secukinumab treatment



PK/PD of an Antibody-Drug: Case Study Secukinumab

Conclusions on Pharmakokinetics

- Substantial levels of secukinumab are observed in skin suggesting the potential for local action.
- Secukinumab ISF distribution into psoriasis lesional and non-lesional skin is similar and is higher than ISF distribution in healthy control skin.

Conclusions on Pharmakodynamics

- Key molecular factors and processes implicated in the pathophysiology of psoriasis were positively impacted in psoriatic skin within 7 days of treatment.
- Secukinumab concentration in skin is sufficient to neutralize IL-17a in psoriatic skin
- Secukinumab affected the expression of a number of pro-inflammatory cytokine

Content

JOANNEUM RESEARCH HEALTH

Bioequivalence
FDA Project

NOVEL METHODOLOGIES AND IVIVC APPROACHES TO ASSESS BIOEQUIVALENCE OF TOPICAL DRUGS

FDA grant: 1U01 FD004946-01

Institute for Biomedicine and Health Sciences
JOANNEUM RESEARCH

Principal Investigator: **Frank Sinner**
Project leader: **Manfred Bodenlenz** and **Katrin Tiffner**

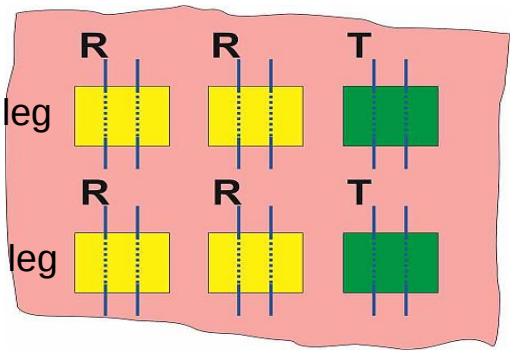
Funding for this project was made possible, in part, by the Food and Drug Administration through grant U01FD004946-01. The views expressed in this abstract do not necessarily reflect the official policies of the Food and Drug Administration, the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

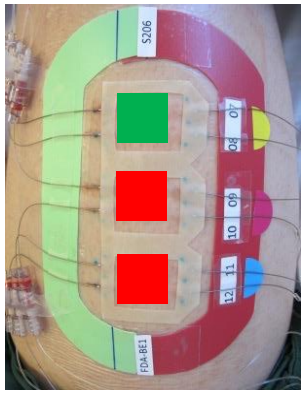
Bioequivalence
Clinical Study

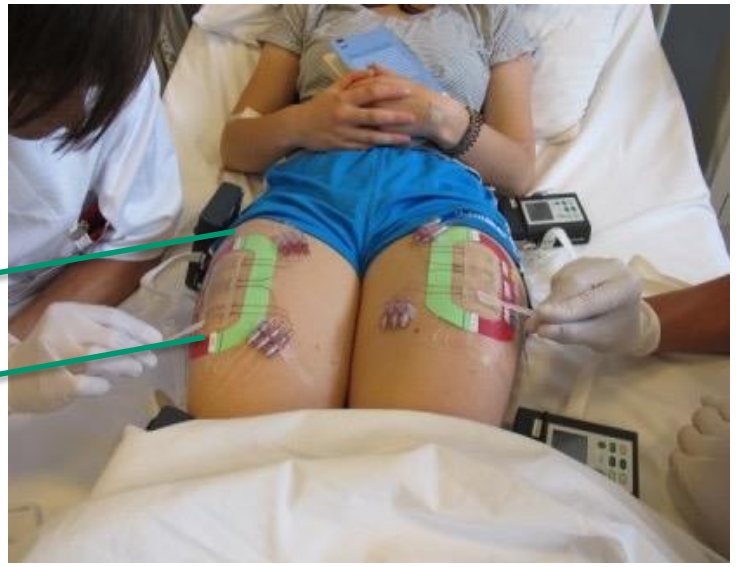
Overall AIM: Investigate the possibility of dOFM to address BE and non-BE of topical formulations in vivo and ex-vivo

Overview Clinical Studies:

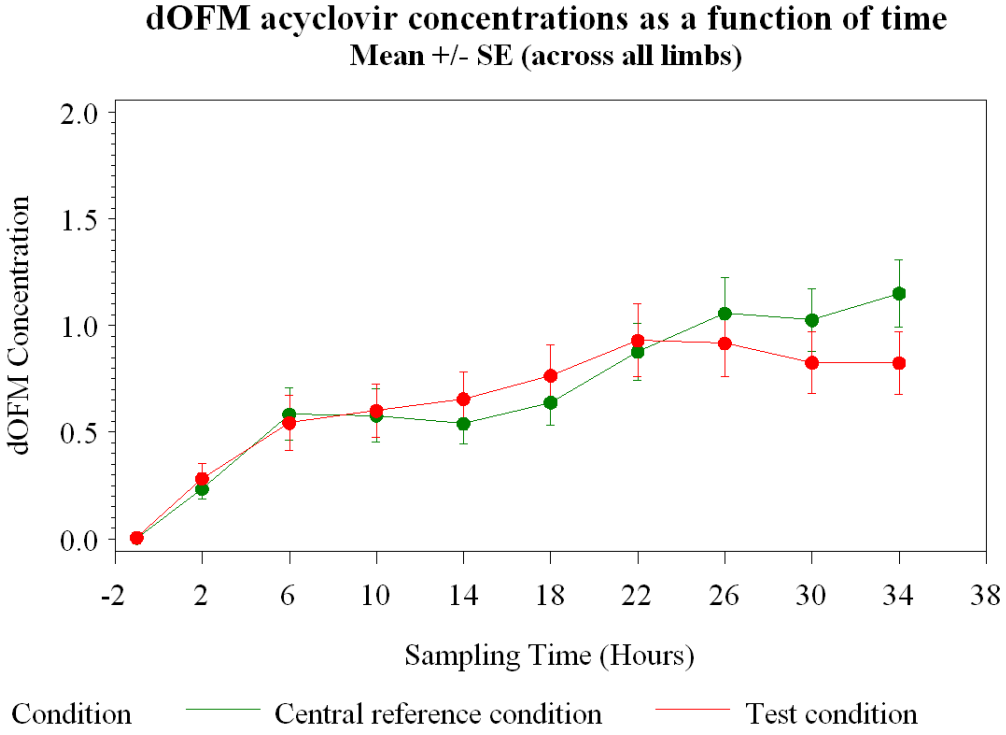
- BE Study in 20 healthy subjects







Clinical Bioavailability
Test versus Reference



Bioavailability: **AUC of Aciclovir A1 are highly reproducible**
AUC of Zovirax US are highly reproducible

Clinical Bioavailability
Test versus Reference

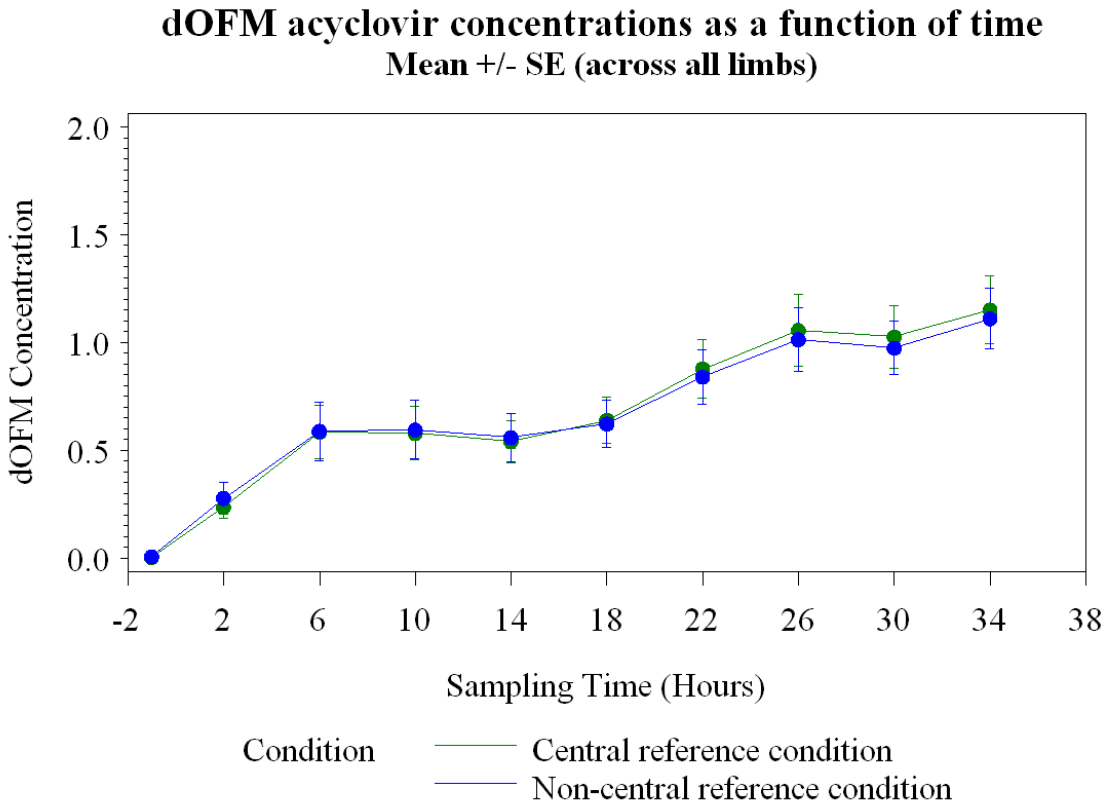
Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.369 ; 0.050] or [69.1 % ; 105.2 %]	[-0.223 ; 0.223] or [80% ; 125%]	x Failed
log(C_{max})	[-0.498 ; 0.022] or [60.8 % ; 102.2%]		x Failed

BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between test and reference condition

BA is established if $CI_{90\%}$ falls within the limits of log(0.8)=-0.223 and log(1.25)=0.223 (cf. FDA Guidance For Industry)

Bioavailability: **BA is different for A1 vs Zovirax US based on AUC**
BA is different for A1 vs Zovirax US based on Cmax

Clinical Bioavailability
Reference versus Reference



Bioavailability: AUC and Cmax of Zoriax US are highly reproducible

Clinical Bioavailability
Reference versus Reference

Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.148 ; 0.162] or [86.2 % ; 117.5 %]	[-0.223 ; 0.223] or [80% ; 125%]	**passed**
log(C_{max})	[-0.155 ; 0.190] or [85.7 % ; 120.9%]		**passed**

BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between the two reference conditions

BA is established if $CI_{90\%}$ falls within the limits of log(0.8)=-0.223 and log(1.25)=0.223 (cf. FDA Guidance For Industry)

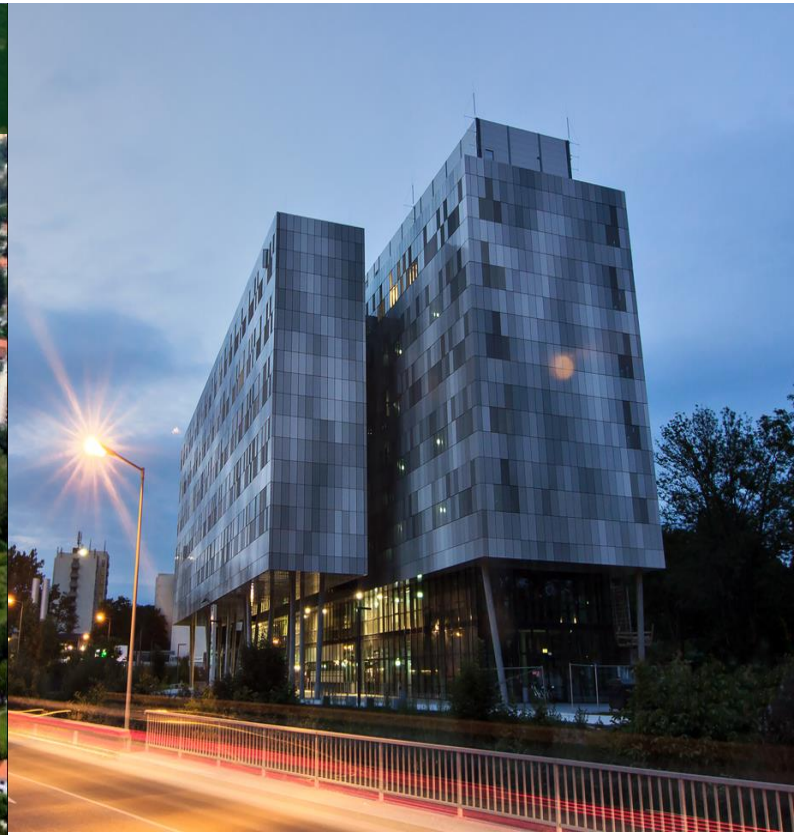
Bioavailability: Same BA for Zovirax US vs Zovirax US based on AUC
Same BA for Zovirax US vs Zovirax US based on Cmax

Content

Close Cooperation

Joanneum Research - Medical University of Graz

58 general wards (~1400 beds)	80,000 patients/year
10 intensive care units (~140 beds)	10,000 patients/year
16 outpatient clinics	500,000 patients/year



One-Stop-Shop
for tissue specific PK and PD

aOFM
adipose-tissue-OFM

cOFM
cerebral-OFM

dOFM
dermal-OFM

EN ISO 9001 | EN ISO 13485:2003 | GLP | Data management | statistic

GCP

Preclinical facilities

Mice, rats, rabbits, pigs, sheep

Clinical Facilities
Phase 1-2

- Fully equipped clinical trial center with 12 beds
  - Study performance according to GCP
  - Located at the Medical University of Graz

Thank you for your attention

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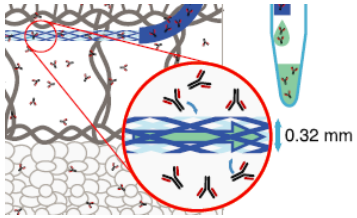

OFM
Peer Reviewed Publications

Case Study Leo Pharma: Two papers in preparation. Submission planned for second half of 2017

Case Study Secukinumab:

1) Secukinumab distributes into dermal interstitial fluid of psoriasis patients as demonstrated by open flow microperfusion.
Exp Dermatol. *2016 Feb;25(2):157-9;* *doi: 10.1111/exd.12863. Epub 2015 Nov 23.*

2) β-Defensin 2 is a responsive biomarker of IL-17A-driven skin pathology in patients with psoriasis.
J Allergy Clin Immunol*. 2017; 139(3):923-932*

3) Secukinumab treatment rapidly leads to positive proteomic and transcriptional changes in psoriatic skin
J. Dermatol. Science, 2016, Volume 84, Issue 1

Case Study Bioequivalence:

4) Open Flow Microperfusion as a Dermal Pharmacokinetic Approach to Evaluate Topical Bioequivalence.
Clin Pharmacokinet. *2017 Jan;56(1):91-98.* *doi: 10.1007/s40262-016-0442-z.*