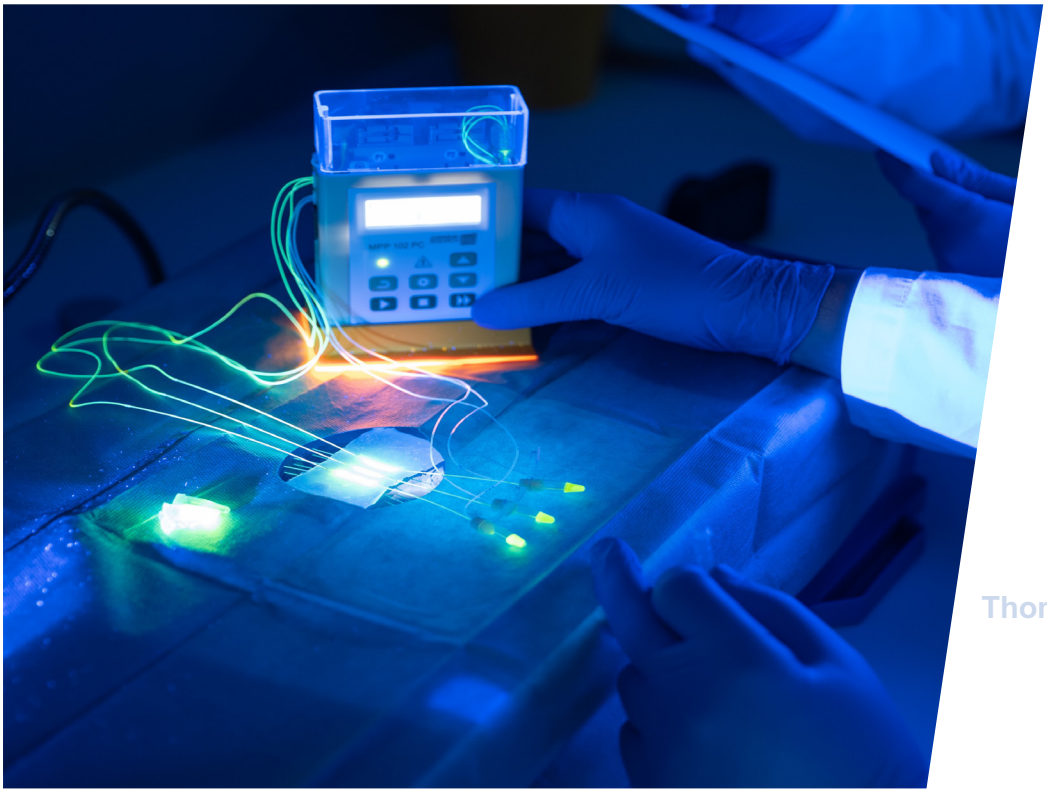

JOANNEUM RESEARCH HEALTH

Webinar

New Ways to Derisk and Accelerate Topical Drug Development

Thomas Birngruber, PhD

Graz, July 12th, 2023

dOFM

Webinar Slides with Speaker Notes

About the webinar

Only one out of 24 drug candidates reaches the market. The key reason for development failure is often a lack of information on drug availability and the drug‘s mode of action in the target tissue. This leads to expensive late-stage clinical trial failures and ultimately to delayed patient benefits. dOFM is a sampling technology that enables continuous monitoring of pharmacokinetics and pharmacodynamics of drugs in skin tissue. This webinar presents preclinical dOFM models and early clinical pilot studies that can be used to provide crucial information predicting the outcome of a main clinical study. The effectiveness of this approach will be demonstrated by presenting selected case studies that illustrate the predictive power of dOFM results for the clinical efficacy of drugs in development. The ability of gaining such insights at the early phase of drug product development is a key factor for pharma companies to follow a „Win Quick Fail Fast” strategy.

Who we are

Today‘s Speaker and Host

Thomas Birngruber, PhD

Deputy Director Joanneum Research HEALTH, Head of Research Group Biomedical Tissue Monitoring

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Wen-Kai Hsiao, PhD

Business Developer at Joanneum Research HEALTH Biomedical Tissue Monitoring

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Speaker: Thomas Birngruber, PhD Deputy Director Joanneum Research HEALTH, Head of Research Group Biomedical Tissue Monitoring

Thomas Birngruber has a background in biomedical engineering, inter-disciplinary medical research and in-vivo sampling techniques. He has more than 15 years of experience with the use of Open Flow Microperfusion (OFM) and other sampling techniques in different tissues (brain, dermal, and adipose tissue). He has led the optimization and standardization of dermal OFM in the last few years.

Speaker: Wen-Kai Hsiao, PhD Business Developer, Biomedical Tissue Monitoring

Wen-Kai Hsiao has a background in mechanical engineering. Prior to joining Joanneum Research HEALTH he worked in a broad range of fields including academia, entrepreneurship, and advanced process development for the pharmaceutical industry.

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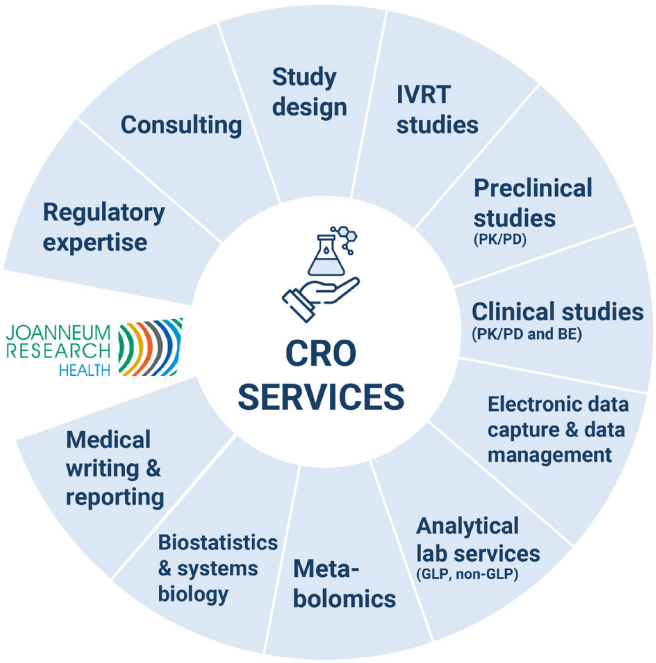


Who we are

We are a boutique CRO for research projects and drug development programs

About the Webinar

5

Key Learnings

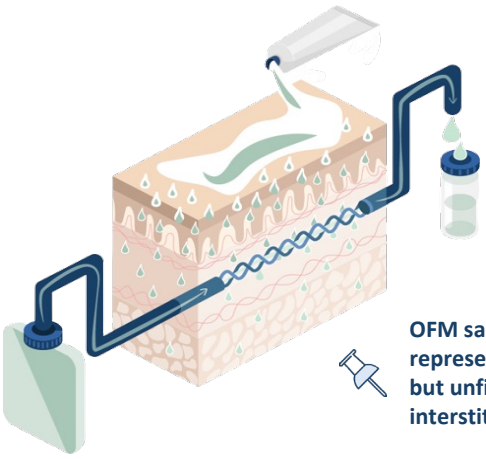
What is dOFM and what is it used for?

What can dOFM sampling contribute at the R&D sweet spot?

What is the difference between a biopsy and a dOFM sample?

How can clinical drug efficacy be predicted using dOFM?

dOFM Working Principle



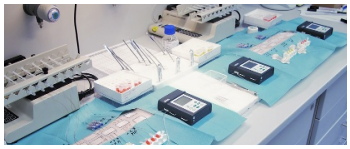
OFM samples represent diluted but unfiltered interstitial fluid.

- Provides highly relevant information from the target tissue.
- Continuous time-resolved monitoring of PK and PD up to 72 hours.
- Sampling of lipophilic substances[1,2], high molecular weight substances[3,4], immune cells, etc.
- Substances can be applied directly into the dermis via dOFM.
- Excellent translatability: same dOFM setup in clinical, preclinical and ex-vivo studies.

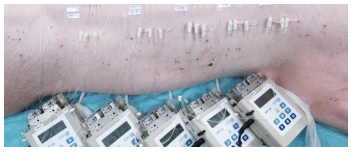
[1] CP-17; logP 3.5 (Bodenlenz et al. 2016) [2] fentanyl; logP 4.5 (Holmgaard et al. 2011) [3] antibodies (Dragatin et al. 2016), [4] cytokines (Kolbinger et al. 2016)

We have developed dOFM - dermal Open Flow Microperfusion, a dermal sampling method that allows continuous sampling of interstitial fluid from the target tissue – in this case the dermis.
The working principle is easy to understand: A dOFM probe is inserted into the dermis and perfused with a constant flow of a physiological fluid.
The exchange area, consisting of a mesh, provides direct contact between the perfusate and the tissue surrounding the dOFM probe.

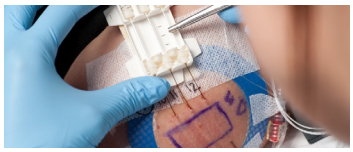
dOFM translational models



Ex-vivo models using animal and human explants



In-vivo animal models



Clinical studies

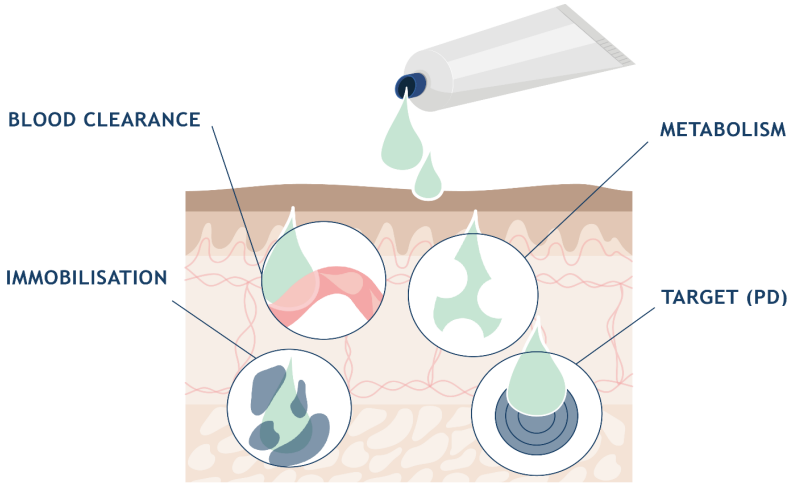
We offer pig and human ex-vivo models of whole-skin explants to determine skin penetration and dermal concentration profiles for drug penetration screening.
In the first step, we are using recently explanted skin or frozen skin and we can clearly see the benefits of fresh non-frozen skin.

In the next translational step we are using in-vivo models such as rats and pigs. These in-vivo models also cover the systemic transport via the blood vessels.
In these models we can also integrate different disease models into experimental designs.

In the third step, we are offering clinical dOFM studies that include healthy volunteers and patients with different skin diseases.

The huge amount of time-resolved data and the highly relevant information from the target tissue provide a unique and very dense data set. This allows a reduction of involved subjects, reduces the study budget and also inter-subject variability.

What happens to the API after topical application?



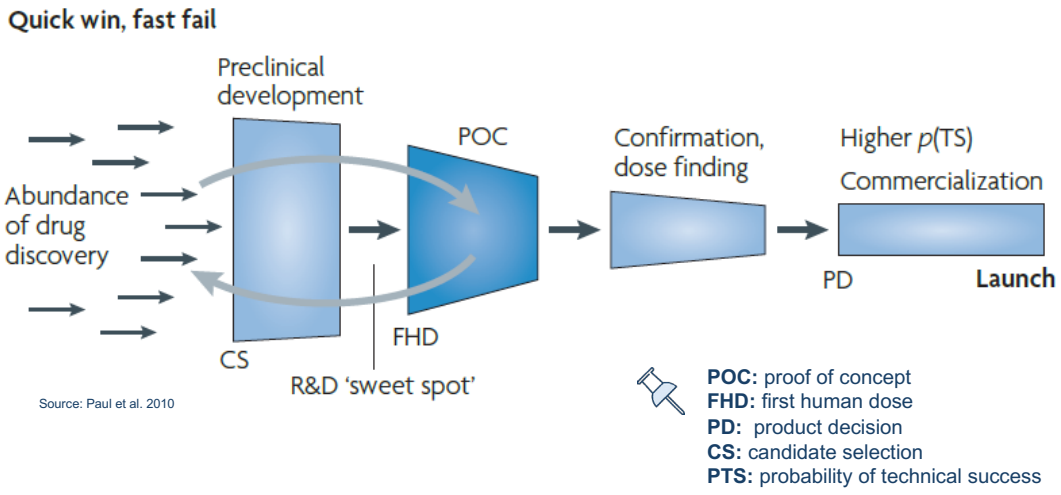
Source: JOANNEUM RESEARCH

A central question that often comes up during drug development is: what happens to the API after topical application:

- Is it released from the formulation?
- Does it cross the skin barrier?
- Is it cleared from the skin via blood flow?
- Is it bound to tissue structures in the skin?
- Is it metabolized into an active or inactive form?
- Or does it bind to the drug target and generate a pharmacodynamic effect?

dOFM study designs allow us to answer all these questions.

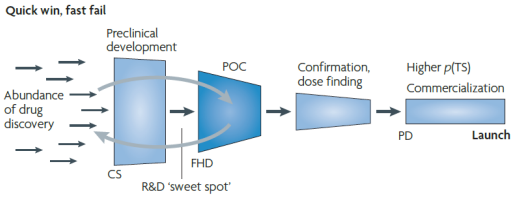
The „Quick Win, Fast Fail“ Paradigm



Many listeners today who work in drug development are aware of the importance of having data available at important decision-making time points.

Already in 2010 Stephen Paul described the quick win, fast fail paradigm in drug development. He defined the so called R&D sweet spot
in the phase after preclinical tests and before the first clinical studies start. At this sweet spot drug development can get very expensive.

The „Quick Win, Fast Fail“ Paradigm



Source: Paul et al. 2010

- At the R&D sweet spot, the development costs increase with GMP, clinical study, etc.
- dOFM derisking has a focus on decision making at this R&D sweet spot.
  - Selection/Testing of Topical Formulations in the Skin
  - Prediction of Clinical Results at the R&D Sweet Spot
  - Early Dose-Response Studies via dOFM Microdosing

Any information that can minimize the risk or derisk processes following the R&D sweetspot will make the drug development process much more efficient.

This R&D sweet spot is the time point at which dOFM has its most valuable application – in the prediction of the outcome of the following clinical studies.

On the next slides I will present examples for:

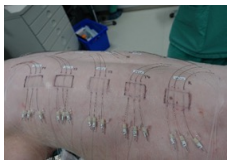
selection and testing of topical formulations in the skin
Prediction of clinical results at the R&D sweetspot
And Early dose-response studies via dOFM microdosing

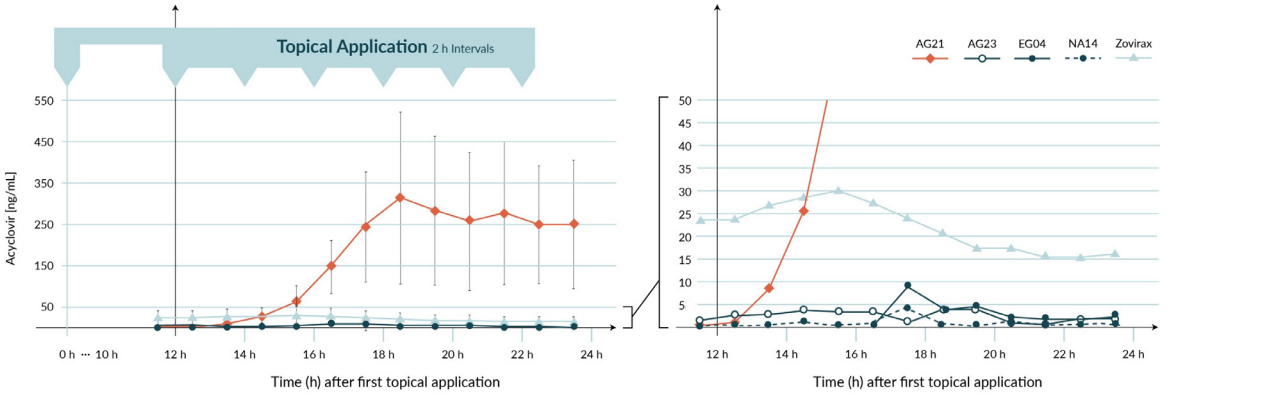
Selection and Testing of Topical Formulations in the Skin

One of the major reasons for failures in the drug development process especially for topical drugs is in pharmacokinetics and not being able to deliver the right amount of API to the target tissue.

Therefore we will start with selection and Testing of topical formulations in the skin.

Direct Comparison of Formulations in One Subject





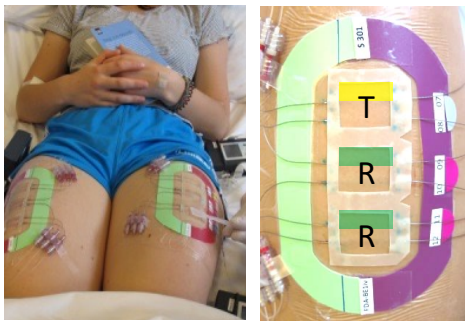
On this slide you can see a typical setup design for direct comparison of different formulations in a preclinical in-vivo pig model
The API in this setup was Acyclovir – a substance that is known for low skin penetration.
We compared Zovirax, a marketed formulation, with other formulations that were under development.

Most of the new formulations showed worse penetration characteristics compared to Zovirax but one formulation, an aqueous gel with 0.2% acyclovir, AG21 here in red showed a remarkably improved acyclovir delivery across the skin barrier into the dermis. In the right diagram the y axes has been magnified so that you can also see that the dynamics of acyclovir in the skin are completely different from all the other formulations and show a highly dynamic increase soon after topical drug application.

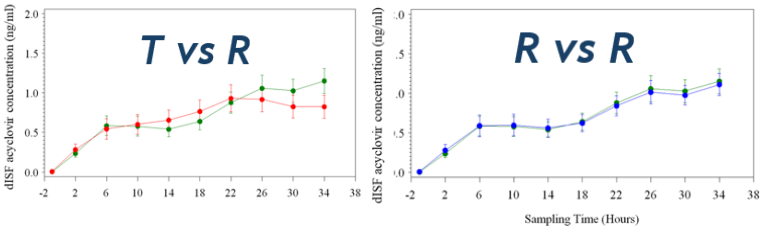
Formulation can be Selected with a Minimal Study Setup

Comparing two marketed acyclovir formulations

- 20 healthy subjects (instead of ~1000 patients in standard endpoint setting)
- Application of multiple drugs on one subject …
  - allows direct comparison
  - reduces inter-subject variability (>80%)



(R) Zovirax vs. (T) Aciclovir 1A Pharma



"Variability of Skin Pharmacokinetic Data" Bodenlenz et al. Pharmaceutical Research. 2020

This slides shows a clinical application of comparing 2 different formulations in healthy volunteers. This study has been conducted in the framework of our very successful scientific collaboration with FDA that has been ongoing for the last 10 years and is still continuing.

As an example I am showing you a study where we have compared the pharmacokinetics of 2 acyclovir formulations one is, like in the previous preclinical study, Zovirax, the other is a comparable product on the Austrian market from 1A Pharma. We compared these two formulations on the highest quality level: We tested for bioequivalence of topical drugs based on pharmacokinetic information directly from the dermis by using OFM sampling.

On the left you can see that 2 OFM sampling setups were placed on one healthy volunteer – one setup on each thigh. In the green application sites, marked with an R (for reference), the same formulation was applied in adjacent application areas
At the same time a test formulation was applied on the yellow application site on the same thigh marked as (T).

A standard bioequivalence test would have required approximately 1,000 patients but using this dOFM approach for bioequivalence testing, only 20 healthy volunteers were needed.
This is possible because the dOFM setup allows the application of different formulations on the same subject and therefore a direct comparison in the same subject and a reduction of inter-subject data variability that accounts for around 80% of the variability in a data set.

On the right side, the two concentration-time profiles show the accuracy of the dOFM results comparing test versus reference formulation and reference formulation against itself – you can see that these two curves are basically identical.

Prediction of Clinical Results at R&D Sweet Spot

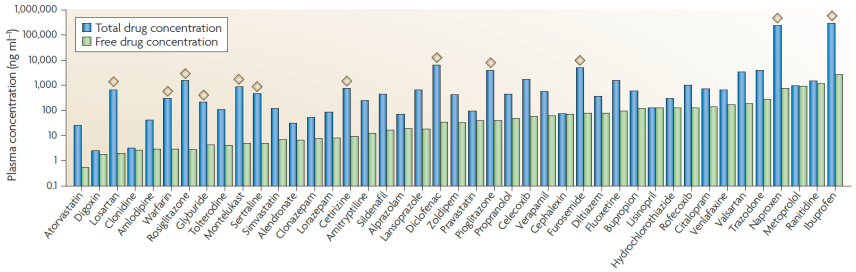
You have just heard how dOFM can be used to select the best formulation and how topical formulation can be tested based on PK concentration-time profiles by using dOFM.

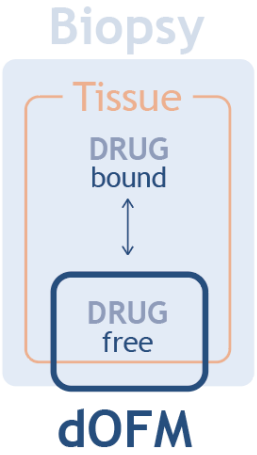
In the next slides I will show you how clinical results can be predicted by using a preclinical dOFM setting at the R&D sweet spot.

Comparison of dOFM and biopsy

Free drug fraction = active drug fraction

"The ADMEa Ecyclopedia – Free Drug Theory" Talevi et al. 2021





"Many of the most prescribed drugs have greater than 98% protein binding" (Smith – Nature Reviews Drug Discovery 2010)

Let me start with a few basics: The dOFM study design is based on the Free Drug Hypothesis which can be used to explain the difference between a dOFM sample and a punch biopsy which is currently often used to investigate PD effects on target tissue level.

The Free Drug Hypothesis is a pharmacological principle that states that only the free drug concentration that is present in the target tissue can trigger a pharmacodynamic effect.

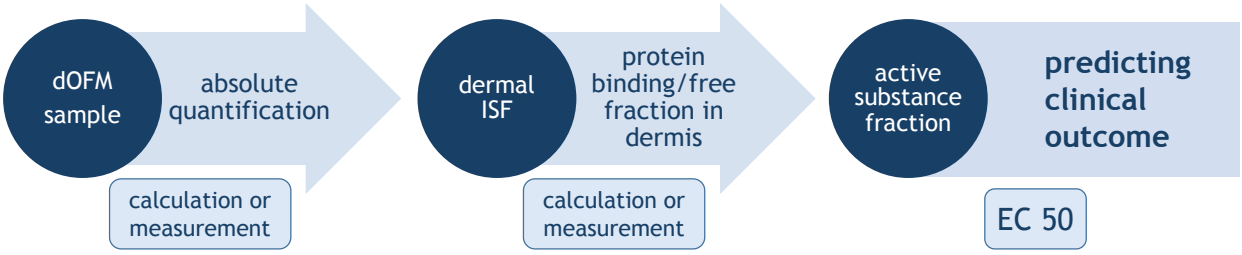
In the figure on left you can see that a majority of the top 100 most prescribed drugs are significantly protein bound which is illustrated by the higher amount of the total drug concentration (in blue) compared to the often much lower free drug concentration (in green). For drugs that are marked with diamonds protein binding exceeds 98%.

This means that a big fraction of the API in the target tissue is bound and thus unavailable to elicit any PD effect according to the Free Drug Hypothesis.

When we only analyze a tissue biopsy we will always have data that reflect both the bound and the free fraction of the drug – as shown in the figure on the right.

dOFM samples on the other hand closely reflect ONLY the free drug fraction in the target tissue and will therefore provide an excellent basis for PD prediction.

Derisking Clinical Study Outcome



This slide shows you a step by step representation of how dOFM can be used to predict clinical outcomes.

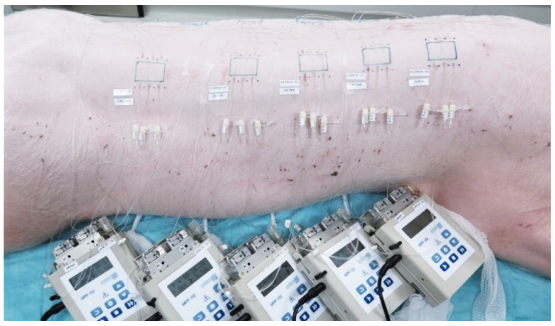
First of all, as I mentioned in the beginning, a dOFM sample reflects the diluted dermal Interstitial fluid. By using either a mathematical calculation or an experimental approach, such as for example a No-net-flux approach, the absolute concentration in the dermal ISF can be estimated.

From the drug concentration in the dermal ISF we can calculate the free/active drug fraction. -This can be done by either estimation or measurement of protein binding in the dermal ISF

The active drug fraction combined with information about the API efficacy (EC50) provides you with a very precise statement predicting the clinical outcome.

Derisking Clinical Study Setups

- ex-vivo human and in-vivo pig studies
  - determination of free fraction in the dermis
  - compare biopsies and dOFM sampling



dOFM

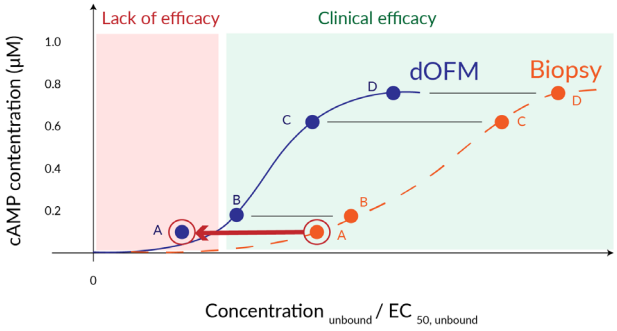
This slide shows you the setups that we use in our de-risking studies.

Depending on the characteristics of the API we either use ex-vivo human dermal tissue and ex-vivo or in-vivo pig experiments.

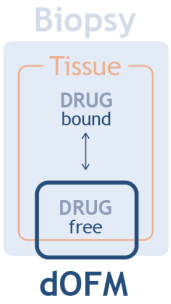
For the ex-vivo setups we use freshly explanted human tissue that has not been frozen and is obtained directly from the operation theater.

Now, let me show you one of our dOFM studies - a show case study where dOFM data have successfully predicted a clinical outcome.

dOFM – not Biopsy - Predicted Clinical Outcome



- dOFM identified ineffective concentration of study drug.
- dOFM concentration correlated with the drugs' clinical efficacies (rank order correlation).
- dOFM showed superior predictivity compared to biopsies.



Results were confirmed in clinical study.

Eirefelt, S., et al. (2020). Evaluating Dermal Pharmacokinetics and Pharmacodynamic Effect of Soft Topical PDE4 Inhibitors: Open Flow Microperfusion and Skin Biopsies. Pharmaceutical Research, 37(12), 243. https://doi.org/10.1007/s11095-020-02962-1

In this study we used ex-vivo human skin to assess the concentration of different APIs directly in the target tissue by using dOFM sampling and punch biopsies. In the diagram on the left the results from dOFM (in blue) and biopsies (in red) for APIs A to D are shown in relation to the clinical effectiveness (displayed on the x-axis) and cAMP levels (on the y-axis) as a measure of target engagement.

The prediction based on biopsy results regarding clinical efficacy is always more optimistic than dOFM results. Especially for Substance A the difference is significant: for substance A biopsies predicted clinical efficacny and dOFM sampling predicted a lack of efficacy. The clinical studies that were performed in parallel confirmed that the dOFM showed superior prediction compared with biopsies.

The whole study was published together with our colleagues from LEO Pharma.

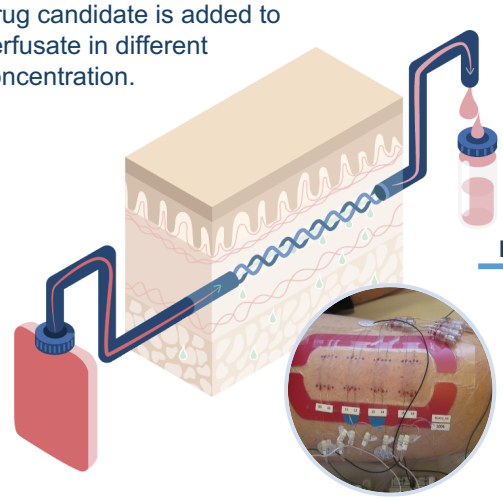
Early Dose-Response Study via dOFM Microdosing

We have covered so far formulation selection and prediction of clinical outcome and are now moving on to the last topic on our list: the possibility of doing early dose-response studies by using dOFM for microdosing in a clinical setting.

dOFM microdosing provides good and stable data on the dose-response of an API.

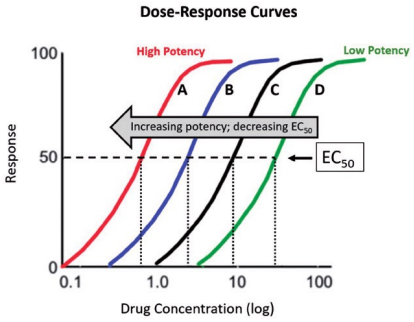
dOFM Microdosing Enables Minimal Systemic Exposure

Drug candidate is added to perfusate in different concentration.



biomarker readout

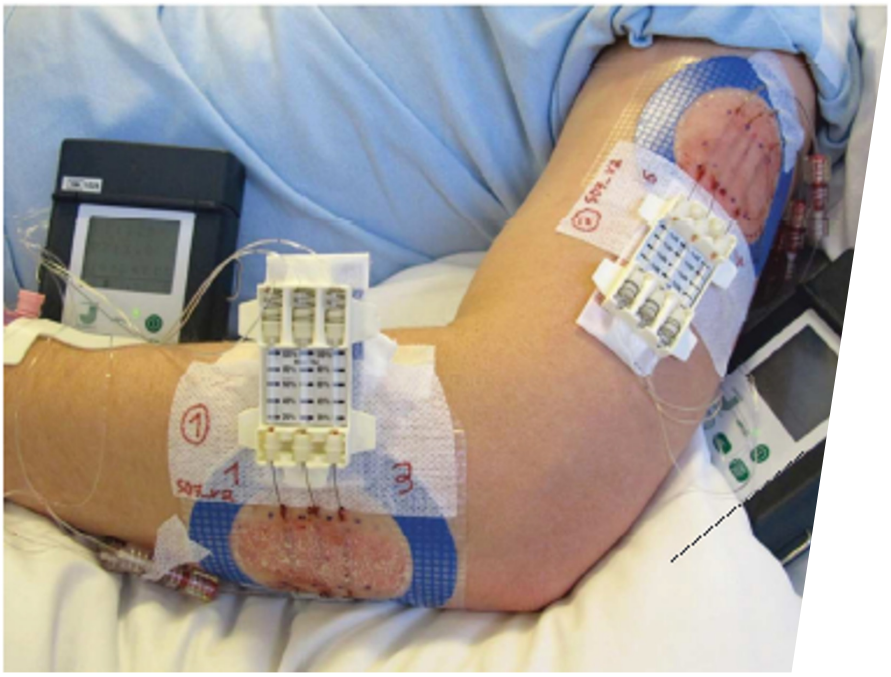
Clinical studies for proof of mechanism + dose response



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

dOFM microdosing is a specific dOFM application for early phase clinical studies where APIs are applied directly into the target tissue via dOFM. This allows you to control exactly how much API is added into the dermis and thus significantly reduces potential systemic side effects.

Another benefit of the dOFM microdosing approach is that you can simultaneously apply multiple doses into one subject in a dose-response study which greatly reduces inter-subject variability. The API effect is monitored via biomarkers that are collected in the same dOFM sampling process that delivers the API into the skin.



NOVARTIS

Show Case: Clinical Study for Systemic Drug: Novartis - Secukinumab

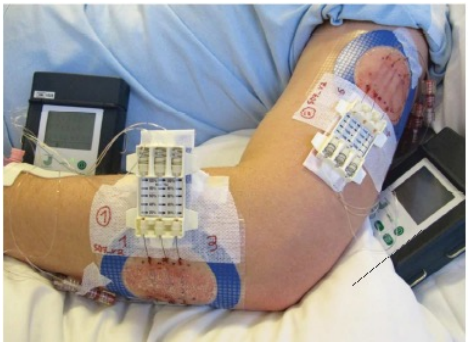
dOFM

And now we are moving on to the last part of the presentation. On the next few slides I will present a clinical study as a show case which highlights several technological features of dOFM sampling that are combined in one clinical study. This study was done for Novartis and the results have been published together with Novartis.

The main aim was to investigate PK and PD of the antibody drug secukinumab in healthy volunteers and patients with psoriasis.

Proof of Concept for Systemic Antibody Drug

(1) Pharmacokinetics of API in human target tissue
(2) Target verification in small but efficient clinical setup
(3) Pharmacodynamics
(4) Target-tissue specific biomarker



Source: JOANNEUM RESEARCH

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

dOFM

This is a short summary of the study design for a proof of concept study to test a systemic antibody drug. The aims of this study were

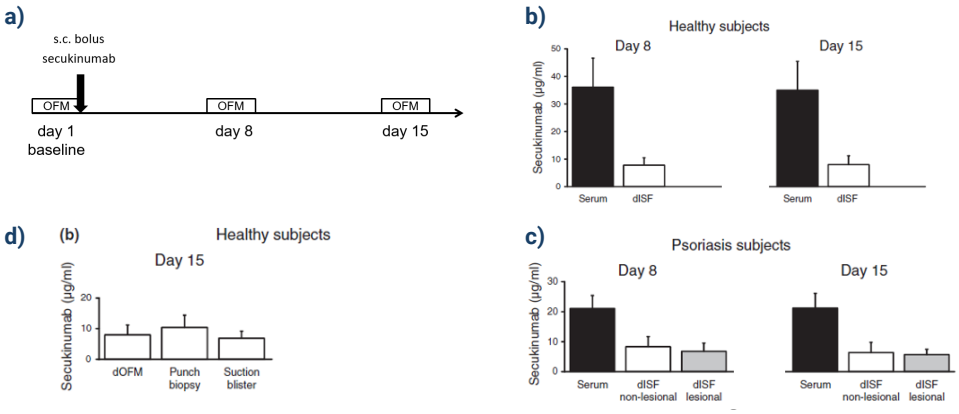
- to assess the pharmacokinetics of the API in the human target tissue, in this case the dermis.
- target verification in a small but very efficient clinical setup.
- to assess pharmacodynamics locally in the skin via dOFM sampling.
- we also screened for target tissue-specific biomarkers.

The study drug was secukinumab, an antibody for the treatment of psoriasis. We included healthy volunteers and psoriasis patients into the study.

Pharmacokinetics were measured in serum and the interstitial fluid (ISF) of healthy skin and lesional skin.

We investigated biomarker pattern in dermal interstitial fluid and the serum.

(1) 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

Let´s look at a few details: The study was done in 8 healthy subjects and 8 psoriasis patients.

Here in the top row on the left in figure a) you can see the basic design. On day one, we performed a baseline sampling. After completing all sampling procedures, study subjects received a subcutaneous injection of secukinumab.
We monitored the absolute concentration of the antibody on day 8 and day 15 in serum and dermal interstitial fluid.

In the figures on the right you can see the pharmacokinetic behavior of secukinumab over the whole study. In the figure on the top you see the data for healthy volunteers: The antibody concentration in the skin (white bar) was substantially lower than the concentration in serum (black bar). You can also see that both concentrations remained very stable from day 8 to day 15.

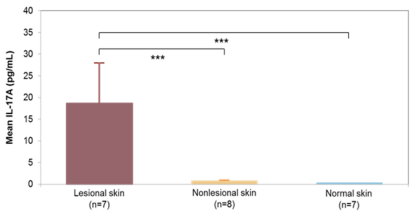
A similar pharmacokinetic behavior was observed in patients with psoriasis (in figure c). Here we took additional samples in lesional skin to compare lesional and non-lesional skin by using dOFM sampling.

In figure (d) we compared different sampling technologies to assess dermal antibody concentration (dOFM, punch biopsy, and blister suction) and all delivered very similar results.

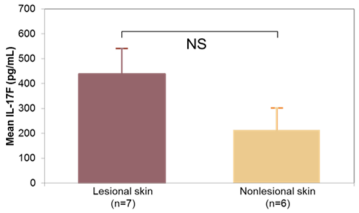
24

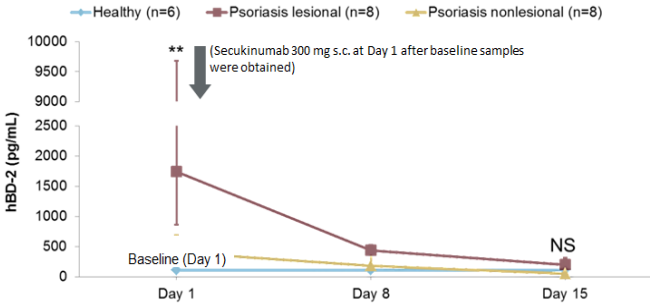
(2) dOFM Data Verified the Therapeutic Target
(3) Pharmacodynamics of Treatment

Therapeutic target (IL-17A)



Therapeutic target (IL-17F)





Monitoring of therapeutic effect based on downstream biomarker of IL-17A (hBD-2)

dOFM

The figures on the left show that we were able to verify IL-17A as a valid therapeutic target and that it was significantly elevated in lesional skin compared with non-lesional skin and skin from healthy volunteers.

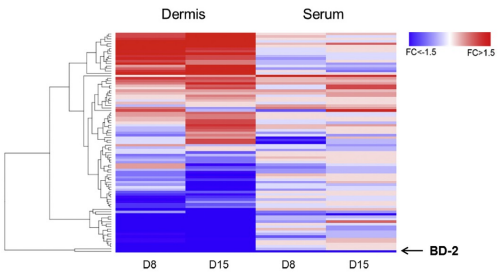
IL-17F in the figure below did not show any significant difference between lesional and non-lesional skin.

In order to monitor pharmacodynamics in the skin we chose beta-defensin as a downstream biomarker for IL-17A. It was not possible to investigate IL-17A directly as it would be bound to the antibody secukinumab after treatment.

In the figure on the right you can see that beta-defensin showed a significant difference between lesional and healthy skin. The difference declined and disappeared over 2 weeks of subcutaneous antibody application.

These findings were also reflected by dermatological observations of disappearing lesions that happened simultaneously.

Target-Tissue-Specific dOFM Provides Increased Sensitivity for Biomarkers



	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

Different signaling pathways in the dermis of patients with psoriasis.

"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

In the same study we also performed a biomarker screening in dOFM samples and serum.

On the left side the heat map shows that tissue-specific biomarkers were by far more pronounced in the dermal interstitial fluid (left column) compared to serum (right column).

Beta-defensin for example was 20 to 30 fold upregulated in the dermal interstitial fluid whereas the measurement in serum only indicated a 3 to 4 fold increase.

This underlines the value of dOFM sampling for skin specific biomarkers.

I hope I was able to demonstrate with this show case study the possibilities that dOFM offers in a clinical setting.

Key Learnings

What is dOFM, and what is it used for?

dOFM (dermal open flow microperfusion) is a sampling technology that enables PK and PD studies on tissue level in clinical and preclinical settings.

What can dOFM sampling contribute at the R&D sweet spot?

A phase during drug development where decisions are very critical. All information that leads to de-risking of the processes following the R&D sweet spot makes the drug development process very efficient.

What is the difference between a biopsy and a dOFM sample?

A biopsy contains a big fraction of bound API that is inactive whereas dOFM contains the free and active drug fraction and is thus highly predictive for the drug effect.

How can clinical drug efficacy be predicted using dOFM?

Preclinical dOFM setups provide a PK-based ranking for candidate formulations. dOFM delivers the required API concentration to the target tissue in clinical microdosing studies.

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