In Vivo Dermal Open Flow Microperfusion: A Novel Approach to Evaluating Topical Bioavailability and Bioequivalence

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FDA approval for topical generic drugs - with some exceptions – requires a Comparative Clinical Endpoint Bioequivalence Study

**Vision: Using dOFM for PK-based Bioequivalence Studies**

<table>
<thead>
<tr>
<th>Patients</th>
<th>In-Vivo</th>
<th>Patients</th>
<th>PK Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hundreds to thousands</td>
<td>Endpoint Study</td>
<td>Healthy subjects</td>
<td>Few weeks</td>
</tr>
<tr>
<td>Several month to years</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Skin PK-based BE approaches

Strengths
1. Provide a direct in-vivo measurement of the rate and extent of the active moiety at or near the site of action in the skin.
2. Evidence indicates that dermal sampling has the potential to differentiate pharmacokinetic profiles by their magnitude.

Challenges
1. Existing sampling methods have limitations.
2. Limited sampling time, often < 8 hours.
3. High variability of skin PK data.
Skin PK-based BE approaches

Open Flow Microperfusion

✔ OFM samples represent diluted but unfiltered interstitial fluid

CE-certified for clinical use
All drugs are accessible in-vivo in the dermis

- **lipophilic substances**
  - Bodenlenz et al. 2016 (CP-17; logP 3.5)
  - Holmgaard et al. 2011 (Fentanyl; logP 4.5)

- **high molecular weight substances (up to cells)**
  - Dragatin et al. 2016 (Quantification of antibodies in skin)
  - Kolbinger et al. 2016 (Cytokines in the skin in healthy & patients)
Skin PK-based BE approaches
Open Flow Microperfusion

✓ dOFM shows dose dependent dermal AUC profiles

Clinical dOFM studies in skin:
Corticoid (topical) – 26 h clinical
Antibody (SC) – 17 h clinical
Skin PK-based BE approaches using dOFM

Strengths

1. Provide a direct in-vivo measurement of the rate and extent of the active moiety at or near the site of action in the skin.
2. Evidence indicates that dermal sampling has the potential to differentiate pharmacokinetic profiles by their magnitude.

Challenges

1. Limitations of existing sampling methods
   → no limitation as dOFM samples diluted ISF
2. Limited sampling time, often < 8 hours
   → no limitation as dOFM samples up to 48 hours
3. High variability of skin PK data
   → optimization of dOFM during the project
Overall AIM: Investigate the capability of dOFM to address BE and non-BE of topical formulations in-vivo.

- Head-to-head comparison within one subject to minimize inter-subject effect on BE.

- Use application-triplets with
  - two separate application sites for reference product → for BE
  - one application site for a non-Q1 product → for non-BE

- Healthy subjects with intact skin integrity for best discrimination of formulations.

- Use a drug for which skin PK was never successfully monitored in healthy subjects.
Variations may result from differences in:

- Hairiness
- Hair shaving
- Sweat duct
- Skin barrier (stratum corneum) properties
- Skin care products use
- Skin condition (e.g. Solarium)
- Room temperature and humidity

✔ Controlling all significantly contributing factors which add data variability - or at least monitoring them.

- not controlled
- subjects are shaved 5 days before dOFM visit
- not controlled
- monitored by TEWL and Impedance
- not allowed 5 days before dOFM visit
- visual check at screening visit
- controlled at 22 ± 1° C ; 40 - 60% rel. humidity
dOFM Optimization

Controlled or Monitored Parameters

✓ Controlling all significantly contributing factors which add data variability - or at least monitoring them.

Variations may result from differences in

- Trauma formation
- Application site
- Dosage application
- Probe depth
- Flow rate
- Local blood flow
- Lateral diffusion and cross-talk
- Systemic absorption and cross-talk

- Universal Parameters
- Drug Dependent Parameters
Minimized trauma formation by cooling.

Variations may result from differences in:

- Trauma formation
- Application site
- Dosage application
- Probe depth
- Flow rate
- Local blood flow
- Lateral diffusion and cross-talk
- Systemic absorption and cross-talk

Standardized by cooling after dOFM insertion
Homogeneous drug application by using an application template.

Variations may result from differences in:

- Trauma formation
- Application site
- Dosage application
- Probe depth
- Flow rate
- Local blood flow
- Lateral diffusion and cross-talk
- Systemic absorption and cross-talk

Standardized by use of application template and Standardization of application
- dOFM probe depth measurement for each probe.

Variations may result from differences in:
- Trauma formation
- Application site
- Dosage application
- Probe depth
- Flow rate
- Local blood flow
- Lateral diffusion and cross-talk
- Systemic absorption and cross-talk

Depth of exchange area measured by ultrasound
Stable flow rate of dOFM probes over 36 hours.

Variations may result from differences in:

- Trauma formation
- Application site
- Dosage application
- Probe depth
- Flow rate
- Local blood flow
- Lateral diffusion and cross-talk
- Systemic absorption and cross-talk

Flow rates of all probes in one subject
Monitoring local blood flow by internal standard in OFM perfusate.

Local blood flow monitoring by loss of glucose from dOFM perfusate

Variations may result from differences in:

- Trauma formation
- Application site
- Dosage application
- Probe depth
- Flow rate
- Local blood flow
- Lateral diffusion and cross-talk
- Systemic absorption and cross-talk

Glucose loss influenced by local blood flow

47% loss, 42% loss, 48% loss, 44% loss
Lateral diffusion and cross-talk

✓ Lateral diffusion for acyclovir is negligible.

Lateral Diffusion between adjacent application sites

\[ R = \frac{|\text{dOFM Samples BLANC SITES} > \text{LLOD}|}{|\text{dOFM Samples US ZOVIRAX SITES} > \text{LLOD}|} \]

Definition: no lateral diffusion if \( R < 0.05 \)

Methodology

- results from all 6 subjects of phase 1
- 10,000 bootstrap estimates were computed
- creation of confidence interval for the true population value of the test statistic \( R \)
- a one-sided 95% confidence interval was constructed

Results

<table>
<thead>
<tr>
<th></th>
<th>MIN</th>
<th>MEDIAN</th>
<th>P90</th>
<th>P95</th>
<th>P99</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.007633588</td>
<td>0.076336</td>
<td>0.10853</td>
<td>0.11831</td>
<td>0.13492</td>
<td>0.18248</td>
</tr>
</tbody>
</table>

US Zovirax
Very high dose of 50 mg/cm²
Test for Systemic Exposure

\[ R = \frac{|\text{Blood Samples} > \text{LLOD}|}{|\text{Total Blood Samples}|} \]

Definition: no systemic exposure if \( R < 0.05 \)

Methodology

- 6 subjects, 6 application sites
- 10,000 bootstrap estimates were computed
- creation of confidence interval for the true population value of the test statistic \( R \)
- a one-sided 95% confidence interval was constructed

Results

<table>
<thead>
<tr>
<th>MIN</th>
<th>MEDIAN</th>
<th>P90</th>
<th>P95</th>
<th>P99</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.012821</td>
<td>0.025641</td>
<td>0.038462</td>
<td>0.051282</td>
<td>0.064103</td>
</tr>
</tbody>
</table>

❌ No systemic exposure and thus no influence on PK of dOFM site.

US Zovirax
Very high dose of 50 mg/cm²
High quality standards are key to reliable skin PK studies.

**Statistical Analysis Plan**

- Software Verification and Validation Report
- OFMLabData Import Validation Plan
- OFMLabData Import Validation Report
- OFMLabData Import SOPs

**Data Management Plan**

- eCRFs
- SDFs

**GCP**

**GLP lab**

- Method Validation Plan
- Method validation Report
- Method SOPs
- Study Analysis Plan
Highly controlled set-up has been developed.

Variations may result from differences in:

- Trauma formation → Controlled by cooling
- Application site → Controlled by application template
- Dosage application → Controlled by standardization
- Probe depth → Monitored by ultrasound
- Flow rate → Monitored by sample weight
- Local blood flow → Monitored by glucose marker
- Lateral diffusion and cross-talk → Negligible
- Systemic absorption and cross-talk → No systemic exposure
Comparative IVRT study

Investigated drugs

- All 5% acyclovir creams investigated.

- Reference product Zovirax cream 5% (GSK, U.S.) was compared against itself and six test products:
  - Zovirax cream 5% (GSK, Vienna, Austria)
  - Zovirax ointment 5% (GSK, U.S.)
  - Aciclostad 5% (STADA, Austria)
  - Aciclovir 1A Pharma Cream 5% (1A Pharma, Austria)
  - Antiviral cold Sore cream 5% (Boots, UK)
  - Zovirax cold Sore cream 5% (GlaxoSmithKline, Brentford, UK)

- Statistical method:
  Mann-Whitney U test according to USP general chapter <1724>
### Comparative IVRT study

**Apparatus qualification**

- **IVRT apparatus qualification was passed successfully.**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>ACCEPTANCE CRITERIA</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercell Variability (Precision)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accuracy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range of variation $V$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pass</td>
<td></td>
</tr>
<tr>
<td>Volume of the cells</td>
<td>$V \leq 0.48 \text{ mL}$ (^1)</td>
<td>0.33 mL</td>
</tr>
<tr>
<td></td>
<td>$\bar{x}_i \in [12 + 0.6 \text{ mL}, 12 - 0.6 \text{ mL}]$ for $1 \leq i \leq 6$ (^4)</td>
<td></td>
</tr>
<tr>
<td>Diameter of the orifice</td>
<td>$V \leq 0.45 \text{ mm}$ (^2)</td>
<td>0.05 mm</td>
</tr>
<tr>
<td></td>
<td>$\bar{x}_i \in [15 + 0.75 \text{ mm}, 15 - 0.75 \text{ mm}]$ for $1 \leq i \leq 6$ (^4)</td>
<td></td>
</tr>
<tr>
<td>Temperature of the</td>
<td></td>
<td>0.23 °C</td>
</tr>
<tr>
<td>receptor medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\bar{x}_i \in [32 + 1 \text{ °C}, 32 - 1 \text{ °C}]$ for $1 \leq i \leq 6$</td>
<td></td>
</tr>
<tr>
<td>Speed of the magnetic</td>
<td></td>
<td>1.77 rpm</td>
</tr>
<tr>
<td>stirrer</td>
<td>$V \leq 12 \text{ rpm}$ (^3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\bar{x}_i \in [600 + 60 \text{ rpm}, 600 - 60 \text{ rpm}]$ for $1 \leq i \leq 6$ (^5)</td>
<td></td>
</tr>
<tr>
<td>Dispensed sampling volume</td>
<td></td>
<td>10.76 µL</td>
</tr>
<tr>
<td></td>
<td>$\bar{x}_i \in [500 + 15 \text{ µL}, 500 - 15 \text{ µL}]$ for $1 \leq i \leq 6$ (^3)</td>
<td></td>
</tr>
</tbody>
</table>
IVRT: drug selection

"A Comprehensive Approach to Qualify and Validate the Essential Parameters of an In Vitro Release Test (IVRT) Method for Acyclovir Cream, 5%" – published online International Journal of Pharmaceutics – OPEN ACCESS

Comparative IVRT study

IVRT method validation for acyclovir was passed successfully.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance Criteria</th>
<th>Passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane Inertness</td>
<td>No acyclovir binding on the membrane: Recovery of 105.5%</td>
<td>✓</td>
</tr>
<tr>
<td>Receptor medium solubility</td>
<td>Solubility &gt; 10 times higher than the maximum acyclovir concentration in the receptor medium observed during the IVRT study</td>
<td>✓</td>
</tr>
<tr>
<td>Linearity</td>
<td>Lowest $R^2 : 0.97$, no outlier</td>
<td>✓</td>
</tr>
<tr>
<td>Precision and Reproducibility</td>
<td>Inter-run variability 5.8%; intra-run variability 4.4%</td>
<td>✓</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Mean release rate increased with increasing acyclovir concentration</td>
<td>✓</td>
</tr>
<tr>
<td>Specificity</td>
<td>Linear regression model (release rate versus product concentration) $R^2 = 0.943$</td>
<td>✓</td>
</tr>
<tr>
<td>Selectivity</td>
<td>IVRT method accurately identify in-equivalent and equivalent acyclovir products</td>
<td>✓</td>
</tr>
<tr>
<td>Robustness</td>
<td>Release rate for temperature and stirring speed variation deviate &lt; 15%</td>
<td>✓</td>
</tr>
<tr>
<td>Recovery</td>
<td>&lt; 10%; no excessive acyclovir depletion</td>
<td>✓</td>
</tr>
</tbody>
</table>
IVRT: drug selection

Comparative IVRT study

Results

✓ IVRT identified different drug release rates.

Diagram showing cumulative amount released (µg/cm²) vs. square root of time (h^{1/2}) for different creams and ointments.
**Clinical Study Details**

- **Test and Reference** are both 5% acyclovir creams but NON-Q1
- **IVRT**: identical release R:R and non identical release T:R

### Equivalence comparison

<table>
<thead>
<tr>
<th></th>
<th>Equivalently</th>
<th>Computed confidence interval</th>
<th>Lower Limit [%]</th>
<th>Upper Limit [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zovirax cream 5% US v. Zovirax cream 5% US</td>
<td>85.7</td>
<td></td>
<td>103.02</td>
<td></td>
</tr>
<tr>
<td>Zovirax cream 5% US v. Aciclovir 1A Pharma Cream 5%</td>
<td>16.27</td>
<td></td>
<td>19.60</td>
<td></td>
</tr>
</tbody>
</table>

**Acceptance limits**: [75%, 133.33%]
Overall AIM: Investigate the capability of dOFM to address BE and non-BE of topical formulations in-vivo.

Overview Clinical Studies:

- 20 healthy subjects
- Reference: Zovirax® US
- Test: Aciclovir-1A Pharma Austria
- 2 application triplets per subject
- 15 mg/cm² cream application
- 36 hours dOFM sampling time
Highly standardized clinical BE study design.

**dOFM BE Study**

**Clinical Study Details**

- High standardized clinical BE study design.
- TEWL by Aquaflux AF200
- Impedance by JOANNEUM
- Ultrasound by GE-Healthcare
✓ All procedures are standardized by using templates and SOPs.
Clinical Bioavailability
Test versus Reference

✓ Bioavailability: AUC and $T_{\text{max}}$ of Aciclovir A1 are highly reproducible
AUC and $T_{\text{max}}$ of Zovirax US are highly reproducible

20 healthy subjects
Clinical Bioavailability
Test versus Reference

- BA is different for Aciclovir 1A vs Zovirax US based on AUC
- BA is different for Aciclovir 1A vs Zovirax US based on C_{max}

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Cl_{90}%</th>
<th>BE-limits</th>
<th>Cl_{90}% within BE-limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(AUC0-36h)</td>
<td>[-0.369 ; 0.050] or [69.1 % ; 105.2 %]</td>
<td>[-0.223 ; 0.223] or [80% ; 125%]</td>
<td>x Failed</td>
</tr>
<tr>
<td>log(C_{max})</td>
<td>[-0.498 ; 0.022] or [60.8 % ; 102.2%]</td>
<td></td>
<td>x Failed</td>
</tr>
</tbody>
</table>

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

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

✓ Bioavailability: AUC and $C_{\text{max}}$ of Zovirax US are highly reproducible.

**dOFM BE Study**

![Graph showing dOFM acyclovir concentrations as a function of time](image)

- **dOFM acyclovir concentrations as a function of time**
  - Mean +/- SE (across all limbs)

- **20 healthy subjects**

![Image of subjects with patches on their legs](image)
Clinical Bioavailability
Reference versus Reference

✔ Same BA for Zovirax US vs Zovirax US based on AUC
✔ Same BA for Zovirax US vs Zovirax US based on \( C_{\text{max}} \)

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>CI(_{90%})</th>
<th>BE-limits</th>
<th>CI(_{90%}) within BE-limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(AUC(_{0-36h}))</td>
<td>[-0.148 ; 0.162] or [86.2 % ; 117.5 %]</td>
<td>[-0.223 ; 0.223] or [80% ; 125%]</td>
<td>passed</td>
</tr>
<tr>
<td>log(( C_{\text{max}} ))</td>
<td>[-0.155 ; 0.190] or [85.7 % ; 120.9%]</td>
<td></td>
<td>passed</td>
</tr>
</tbody>
</table>

BA is tested for the difference of the log-transformed outcome variables (AUC, \( C_{\text{max}} \)) 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).
Skin penetration insights

Total variability

BE study set-up shows low intra-subject variability.

Total CV$_{\log\text{AUC}_{\text{Acyc}}}$ was 39% - 44%

Components of total CV (ANOVA):

- Inter-subject variability: 84-91% OFM
  (61% Microdialysis Benfeldt et al.)
- Intra-subject variability: 9-16% OFM
  (39% Microdialysis Benfeldt et al.)

logAUC Zovirax® 84% 9% 16% 7%

logAUC Aciclovir 1A Pharma 91% 4% 9% 5%

logAUC lidocaine MD (Benfeldt et al.) 61% 20% 19% 39%

Inter-subject variability has
- a strong correlation with skin impedance (Joanneum®) (p=0.69-0.75, p<0.001)
- a weak correlation with TEWL (p=0.29-0.37, n.s)
- no influence on BE in head-to-head design

Intra-subject variability has
- a weak correlation with skin temperature (correlation analysis: r=0.25, p<0.05)
- influence on BE in head-to-head design
- deviations of 100-500% between probes within sites - also published for MD
Skin penetration insights

Intra-subject distribution

✓ Is intra-subject variability really due to dOFM?

Hypothesis:

Local skin shunts (follicles, glands) rather than OFM cause majority of intra-subject variability

OFM errors ≤ 10% (also for MD, see Kreilgaard et al. 2001)
Skin penetration insights

Skewed skin penetration pattern

✓ Skin shunts may lead to skewed distribution

- **Ideal homogenous intact skin**
  - IVPT area (=large)
  - OFM area (=small)

- **Small skin impaires**

- **Large skin impaires**

(Particularly) relevant for drug which are bad penetrators.

Acyclovir dOFM AUCs within subjects are **log-normal** distributed.

AUCs standarized in each subject by indiv. mean - **non-normal!**

logAUCs standarized by indiv. mean in each subject - **normal!**

**Skin penetration insights**

*Skewed intra-subject data*
Skin penetration insights

Impact of skewed distribution on BE calculation

✓ Geometric mean is best for skewed distributed acyclovir data

**Arithm.** Mean curve, thereof AUC (published):
BE ✓ - good

| Label | Estimate | Standard Error | Df | t-Value | Pr>|t| | alpha | Lower limit | Upper limit |
|-------|----------|----------------|----|---------|------|-------|-------------|------------|
| R₂ vs. R₁ | 100.7% Δ 0.7% | 109.6% | 39 | 0.07 | 0.9428 | 0.1 | 86.2% | 117.5% |
|        | 90% CI width: 31.3% |

**Geom.** Mean curve, thereof AUC
BE ✓ - better!

| Label | Estimate | Standard Error | Df | t-Value | Pr>|t| | alpha | Lower limit | Upper limit |
|-------|----------|----------------|----|---------|------|-------|-------------|------------|
| R₂ vs. R₁ | 99.7% Δ 0.3% | 108.8% | 39 | -0.03 | 0.9741 | 0.1 | 86.5% | 115.0% |
|        | 90% CI width: 28.5% |
Pharmacokinetics-Based dOFM

Summary

dOFM in-vivo

- is a reproducible, accurate and sensitive method.
- shows very low method-variability.
- reflects in-vivo skin penetration in dermis.
- gives advanced skin penetration insights.

dOFM in-vivo

- can be used to investigate BE on a pharmacokinetic basis.
- could be a useful tool to conduct clinical bioequivalence studies in a low number of healthy subjects.
- is a potential tool to reduce time and costs of clinical bioequivalence studies.

This presentation shows the status of our current work and may not represent final conclusions.
Clinical OFM study A: In-Depth Identification of Influencing Factors of Skin Penetration - Moderate Lipophilic/Protein Bound Drugs

- Pilot (n=6): systemic adsorption and cross-talk; lateral diffusion and cross-talk, sample time for $C_{\text{max}}$ and $\frac{3}{4}$ of AUC
- Main study (n=38): investigate BE of (a) RLD to itself, (b) approved generic product to RLD, (c) non-BE product to RLD, (d) BE identify influencing factors

→ Optimization of screening and OFM BE study design

Clinical OFM study B: Standardized BE Study - Highly Protein Bound Drug

- Pilot (n=6): systemic adsorption and cross-talk; lateral diffusion and cross-talk, sample time for $C_{\text{max}}$ and $\frac{3}{4}$ of AUC
- Main study (n=20): investigate BE of (a) RLD to itself, (b) approved generic product to RLD, (c) non-BE product to RLD

→ Validate OFM as an universal tool for BE studies for topical drugs
A big Thanks to...

Katrin Tiffner  
IVRT and dOFM ex-vivo

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Clinical dOFM BE Study

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Analytics

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Clinical PI

Isadore Kanfer  
BE Consultant Expert

Sam G. Raney  
FDA Project Officer

Bernd Tschapeller  
Data Management

Thomas Augsutin  
Statistics

Thomas Birngruber  
OFM Group Leader

More than 20 other persons

Priyanka Ghosh  
Bryan Newman

Elena Rantou  
Youngsook Lee

Lisa Ko  
Jill Coker

and other....
Thank you for your attention

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