Evaluating Topical Bioavailability In-Vivo: ...
Dermal Open Flow Microperfusion and Equivalence Testing by IVRT

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What dOFM Adds to Pharmacokinetics-Based BA Approaches
Predictive Bioavailability

The Big Picture

**In-Vivo**
- Endpoint Study
- Biomarker Study
- PK Healthy Subjects

**Ex-Vivo**
- Healthy Human Skin

**In-Vitro**
- e.g. IVRT, IVPT

**Physico-chem. Prop.**

Q1 Q3
Q2
Predictive Bioavailability
*The Big Picture*

**In-Vivo**
- Endpoint Study
  - Healthy Human Skin

**Ex-Vivo**
- Healthy Human Skin

**In-Vitro**
- e.g. IVRT, IVPT

**In-Vivo**
- Biomarker Study
- Physico-chem. Prop.
Pharmacokinetics-Based BA Approaches

Dermal Microdialysis (dermal MD)

✔ MD samples represent diluted and filtered interstitial fluid

**dMD has been used for topical BA:**
Benfeldt *JID* 2007 (Lidocaine, 5 h)
Tettey-Amlalo *EurJPharmSci* 2009 (Ketoprofen, 5 h)
Incebayir *PharmRes* 2011 (Oxytetracycline, 4 h)
García Ortiz *SkinPharmPhysiol. 2011* (Metronidazole, 5 h)
Why is dermal MD not accepted by FDA today?

**Strengths**

1. Provides 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 MD has the potential to differentiate pharmacokinetic profiles by their magnitude.

**Limitations**

1. Limitations linked to membrane, e.g. pore size and adsorption
2. Limited sampling time, often < 8 hours
3. Various factors contribute to data variability
OFM samples represent *diluted but unfiltered* interstitial fluid.

CE-certified for clinical use.
Open Flow Microperfusion

✓ Limitation 1 solved: all drugs are accessible in-vivo in the dermis

CE-certified for clinical use
Open Flow Microperfusion

☑️ Limitation 2 solved: In-vivo sampling in the dermis up to 48 hours

**dOFM used for PK-PD in skin:**

- Acyclovir (topical) – 36 h clinical
- Corticoid (topical) – 26 h clinical
- Antibody (SC) – 17 h clinical
- Acyclovir (topical) – 36h ex-vivo human skin
- NCE (topical) – 24 h ex-vivo human skin
Continuous dermal ISF sampling
Sources of Variability

variability due to sampling site

- Differences in skin structure
  - Between subjects
  - Parts of the body
- Hairiness
- Sweat ducts
- Day/night rhythm of local blood flow
- Hair shaving
- Skin care products use
- Skin condition (e.g. solarium)

variability due to methods

- Trauma formation (OFM/MD)
- Dosage application
- Probe depth (OFM/MD)
- Flow rate (OFM/MD)
- Local blood flow (OFM/MD)
- Lateral diffusion
- Systemic diffusion
- Room temperature and humidity
Continuous dermal ISF sampling

Sources of Data Variability

Variability due to sampling site

- Control all significantly contributing factors that add to data variability
- Factors that cannot be controlled are monitored

Variability due to methods

- Differences in skin structure
- Between subjects
- Parts of the body
- Hairiness
- Sweat duct
- Day/night rhythm of local blood flow
- Hair shaving
- Skin care products use
- Skin condition (e.g. solarium)

- Probe depth (dOFM)
- Flow rate (dOFM)
- Local blood flow
- Lateral diffusion
- Systemic diffusion
- Room temperature and humidity

Pharmacokinetics-Based BA Approaches
New dOFM probe
- 0.5 x 15 mm sampling mesh
- patent granted
- use of up to 48 hours

dOFM pump
- portable
- 0.1 – 10 µl/min
- Sterile fluidic kit
- operates 3 OFM probes

CE certified for clinical use
All dOFM procedures are highly standardized
dOFM provides a stable flow rate for 36 hours
dOFM

(3) Performance Verification

✓ dOFM is used to sample analytes for 36 hours
Method Validation for Acyclovir Test for Systemic Exposure

- No systemic exposure
- No influence on PK at dOFM site

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

- No systemic exposure if \( R < 0.05 \)

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>R</td>
<td>0</td>
<td>0.013</td>
<td>0.256</td>
<td>0.039</td>
<td>0.051</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Methodology

- 6 subjects
- 10,000 bootstrap estimates
- Confidence interval created for true population value of test statistic R
- One-sided 95% confidence interval
Method Validation for Acyclovir
Test for Lateral Diffusion

✓ Negligible lateral diffusion in a few cases after 24 h
✓ No significant influence on PK at adjacent dOFM sites

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

no lateral diffusion if \( R < 0.05 \)

Results

<table>
<thead>
<tr>
<th>( R )</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>0.008</td>
<td>0.076</td>
<td>0.109</td>
<td>0.118</td>
<td>0.135</td>
<td>0.183</td>
</tr>
</tbody>
</table>

Methodology

- 6 subjects
- 10,000 bootstrap estimates
- Confidence interval created for true population value of test statistic \( R \)
- One-sided 95% confidence interval
Method Validation for Acyclovir dOFM Probe Depth

✓ Uniform probe depth

Monitoring of probe depth along the whole exchange area

Stratum Corneum

dOFM probe

 probe depth S206

- Integrated depth [mm]
- min [mm]
- max [mm]
Local blood flow monitoring

**Glucose** was used as an internal standard in OFM perfusate

**Cooling** was used to
- reduce local blood flow
- lower glucose loss from perfusate

6 dOFM probes in one subject
Controlled and Monitored Factors

- Hairiness
  - not controlled
- Hair shaving
  - subjects are shaved 5 days before dOFM visit
- Sweat ducts
  - not controlled
- Skin permeation behaviour
  - monitored by TEWL and impedance
- Skin products use
  - not allowed 5 days before dOFM visit
- Skin condition (e.g. Solarium)
  - visual check at screening visit
Controlled by cooling
Controlled by application template
Controlled by standardization
Monitored by ultrasound
Monitored by sample weight
Monitored by glucose marker
Negligible
No systemic exposure
Controlled 22 ± 1°C & 40 - 60% RH

Trauma formation (OFM/MD)
Application site
Dosage application
Probe depth (OFM/MD)
Flow rate (OFM/MD)
Local blood flow (OFM/MD)
Lateral diffusion
Systemic diffusion
Room temperature & relative humidity

Limitation 3 solved: In-vivo variation significantly reduced

variability due to methods
All dOFM procedures are highly standardized

GCP

Data Management Plan

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

GLP lab

Method Validation Plan
Method Validation Report
Method SOPs
Study Analysis Plan

Statistical Analysis Plan
Clinical study in healthy subjects (n=20)

**Reference:** Zovirax cream 5% (US)

**Test:** Aciclovir 1A Pharma Cream 5% (Austria)

**Aims:**
- Investigate BA for \( R \) vs \( R \) for 36 h post-dose
- Investigate BA for \( T \) vs \( R \) for 36 h post-dose
**Clinical Bioavailability**

**Clinical BA Set-Up**

- **SOP controlled clinical BA protocol**

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**Pharmacokinetics-Based BA Approaches**
Clinical Bioavailability
Test versus Reference

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

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

![Graph showing dOFM acyclovir concentrations as a function of time with two conditions: Central reference condition and Test condition.](image)
Bioavailability: BA is different for Aciclovir A1 vs Zovirax US based on AUC
BA is different for Aciclovir A1 vs Zovirax US based on C_{max}

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>$\text{Cl}_{90%}$</th>
<th>BE-limits</th>
<th>$\text{Cl}_{90%}$ within BE-limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(AUC0-36h)</td>
<td>[-0.369 ; 0.050]</td>
<td>[-0.223 ; 0.223]</td>
<td>x Failed</td>
</tr>
<tr>
<td></td>
<td>or [69.1 % ; 105.2 %]</td>
<td>[80% ; 125%]</td>
<td></td>
</tr>
<tr>
<td>log($C_{max}$)</td>
<td>[-0.498 ; 0.022]</td>
<td>or [60.8 % ; 102.2%]</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 $\text{Cl}_{90\%}$ 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 $C_{max}$ of Zoriax US are highly reproducible

**dOFM acyclovir concentrations as a function of time**

Mean +/- SE (across all limbs)

**Condition**
- Central reference condition
- Non-central reference condition
Clinical Bioavailability
Reference versus Reference

✓ Bioavailability: 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>$\text{Cl}_{90%}$</th>
<th>BE-limits</th>
<th>$\text{Cl}_{90%}$ within BE-limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(AUC0-36h)</td>
<td>[-0.148 ; 0.162]</td>
<td>or [-0.223 ; 0.223]</td>
<td>passed</td>
</tr>
<tr>
<td></td>
<td>[86.2 % ; 117.5 %]</td>
<td>or [80% ; 125%]</td>
<td></td>
</tr>
<tr>
<td>log($C_{\text{max}}$)</td>
<td>[-0.155 ; 0.190]</td>
<td>or [-0.223 ; 0.223]</td>
<td>passed</td>
</tr>
<tr>
<td></td>
<td>[85.7 % ; 120.9%]</td>
<td>or [80% ; 125%]</td>
<td></td>
</tr>
</tbody>
</table>

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

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

Pharmacokinetics-Based BA Approaches
Clinical Bioavailability

Influence of Probe Depth on AUC of Acyclovir

✓ dOFM acyclovir concentration does not correlate with probe depth

Pharmacokinetics-Based BA Approaches
Clinical Bioavailability

Correlation of TEWL with AUC of Acyclovir

✓ dOFM acyclovir concentration correlates with TEWL
Clinical Bioavailability

Power Calculation to Show BA

✓ 17 subjects are sufficient to show BA for acyclovir when using dOFM

Probability to show BA as a function of sample size

<table>
<thead>
<tr>
<th>Probability to show BA</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>0.2</td>
<td>11</td>
</tr>
<tr>
<td>0.3</td>
<td>12</td>
</tr>
<tr>
<td>0.4</td>
<td>13</td>
</tr>
<tr>
<td>0.5</td>
<td>14</td>
</tr>
<tr>
<td>0.6</td>
<td>15</td>
</tr>
<tr>
<td>0.7</td>
<td>16</td>
</tr>
<tr>
<td>0.8</td>
<td>17</td>
</tr>
<tr>
<td>0.9</td>
<td>18</td>
</tr>
<tr>
<td>1.0</td>
<td>19</td>
</tr>
</tbody>
</table>

Pharmacokinetics-Based BA Approaches
Predictive Bioavailability

The Big Picture

In-Vivo

Endpoint Study

Biomarker Study

In-Vivo

Physico-chem. Prop.

Ex-Vivo

Healthy Human Skin

In-Vitro

e.g. IVRT, IVPT

Q1 Q2 Q3
### IVRT Method Description

- **Apparatus:** Hanson vertical diffusion cells (VDC, volume: 12 mL, orifice: 15 mm)
- **Receptor medium:** 0.9% saline solution (degassed)
- **Sampling:** 0.5, 1, 2, 3, 4, 5 and 6 hours after dosing
- **Membrane:** Tuffryn® membrane (25 mm, 0.45 μm)
- **Stirring speed:** 600 rpm
- **Temperature:** 32°C
Results

<table>
<thead>
<tr>
<th>Test</th>
<th>Pass</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1: Environmental conditions</td>
<td>✔️</td>
</tr>
<tr>
<td>P2: Capacity of the cells</td>
<td>❌</td>
</tr>
<tr>
<td>P3: Diameter of the orifice of the cell</td>
<td>✔️</td>
</tr>
<tr>
<td>P4: Temperature of the receptor medium</td>
<td>✔️</td>
</tr>
<tr>
<td>P5: Speed of the magnetic stirrer</td>
<td>✔️</td>
</tr>
<tr>
<td>P6: Dispensed sampling volume</td>
<td>✔️</td>
</tr>
</tbody>
</table>

9.77 mL instead of 12 mL

Methodology

Test of VDC apparatus for consistent operation within established limits and tolerances.

✓ Successful qualification of laboratory and IVRT apparatus

IVRT (1) Apparatus Qualification
IVRT (2) Performance Verification

Successful performance verification

Methodology

- IVRT study was conducted according to the USP general chapter <1724>
- 1% hydrocortisone cream (BP 1% w/w; LycorTM 1%, Micro Labs Limited, Bangalore, India)

Results

- **Perfect sink conditions:** Acyclovir solubility > 10 times maximum receptor medium conc. observed during the IVRT study
- R² values range were [0.95 - 1] for all calculated release rates >0.9
- Blank samples before start from each cell showed no acyclovir carry over
- The **inter-run CV** (12.7%) and **intra-run CV** (6.8-10.2%) < 15%
- x₈ = 1.04 and x₂₉ = 1.32 meet the 75% - 133.33% limits of the USP general chapter <1724>

IVRT - in-vitro release testing
IVRT

(3) Method Validation for Acyclovir

✓ IVRT was successfully validated for acyclovir
✓ Tests for selectivity, sensitivity and specificity are ongoing

Results
✓ Membrane inertness: Recovery of 105.5%
✓ Receptor solubility test: Solubility > 10 times maximum receptor medium conc. observed
✓ Linearity: Lowest $R^2$ value was 0.97, no outlier
✓ Precision: Inter-run variability 5.8 %; intra-run variability 4.4 %
✓ Recovery: < 10%, i.e. no excessive acyclovir depletion
✓ Robustness: Release rate for temperature and stirring speed variation deviate < 15%
✗ Sensitivity, specificity and selectivity: ongoing

Methodology
■ IVRT study was conducted according to the USP general chapter <1724> and HPLC-UV method validation according to ICH Q2
■ Validation of the IVRT method for acyclovir (Zovirax cream 5% - GSK, AT)
Results

- Reference versus Zovirax ointment 5% (US) - Non-BE
- Reference versus Zovirax cream 5% (Austria) - Non-BE
- Reference versus Zovirax cold sore cream 5% (GSK, UK) - Non-BE
- Reference versus Aciclostad cream 5% (Austria) - Non-BE
- Reference versus Aciclovir 1A Pharma cream 5% (Austria) - Non-BE
- Reference versus Antiviral cold sore cream 5% (Boots, UK) - Non-BE
- Reference versus Zovirax cream 5% (US) - BE

Methodology

- IVRT study was conducted according to the USP general chapter <1724>
- Pairwise comparison tests Reference versus Test

IVRT Comparative Study

✓ All test products were non-bioequivalent relative to Zovirax US
✓ Zovirax US was bioequivalent to itself
All test products were non-bioequivalent relative to Zovirax US.

- Zovirax US was bioequivalent to itself.
- Non-Zovirax Group (Aciclostad, Aciclovir A1) shows similar behavior.
- Zovirax Group (Zovirax AT and UK) shows similar behavior.
- Non-Zovirax Group shows higher release rates than Zovirax group.

R-Square:
- 1A Pharma: 0.9940
- Aciclostad: 0.9980
- UK Zovirax: 0.9979
- AT Zovirax: 0.9991
- US Zovirax: 0.9968

Bars indicate standard deviation (SD).
In-Vitro In-Vivo Correlation

**Summary**

**In-Vivo**
- dOFM PK profiles of all products are quantifiable for 36 hours
- Similar rate and extent of bioavailability: Zovirax US vs Zovirax US
- Different rate and extent of bioavailability: Aciclovir A1 vs Zovirax US

**IVRT**
- Acyclovir A1 Pharma, Aciclostad > Zovirax UK, AT > Zovirax US
- Similar release rate: Zovirax US vs Zovirax US
- Different release rate: Zovirax US versus all other products
Predictive Bioavailability

The Big Picture

In-Vivo

Endpoint Study

Biomarker Study

Ex-Vivo

Healthy Human Skin

In-Vitro

e.g. IVRT, IVPT

In-Vivo

Physico-chem. Prop.
Ex-Vivo

*dOFM Study Approach*

- Ex-vivo study in excised skin from healthy subjects (n=40)
- *Reference*: Zovirax cream 5% (US)
- *Test*: Aciclovir 1A Pharma Cream 5% (Austria)
- Aims:
  - Investigate BA for R vs R for 36 h post-dose
  - Investigate BA for T vs R for 36 h post-dose

Pharmacokinetics-Based BA Approaches
dOFM

- is highly standardized and reflects the in-vivo skin PK profile
- is able to sample lipophilic and large molecules (up to antibodies) up to 36 hours

dOFM

- showed usability to reflect in-vivo PK differences of topical acyclovir drugs
- proved usability to investigate rate and extent of bioavailability

dOFM may add…

- to In-Vitro In-Vivo Correlation (IVIVC)
- strong support to skin penetration modeling
- the possibility to determine BA in-vivo

….to Pharmacokinetics-Based BA Approaches
Many thanks also to **Mike Roberts** (Princess Alexandra Hospital, Brisbane, Australia) and **Chris Anderson** (Region Östergötland, Sweden) for great scientific discussions.
Thank you for your attention

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There is a Method Available to Assess In-Vivo PK in Dermis

- **In-Vivo**
  - Endpoint Study
  - Biomarker Study
  - PK Healthy Subjects

- **Ex-Vivo**
  - Healthy Human Skin

- **In-Vitro**
  - e.g. IVRT, IVPT

**dOFM**

**Q1**

**Q2**

**Q3**