New Models in the Validation Pipeline for ACD Hazard Testing

human Cell Line Activation Test: h-CLAT

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Approach for Developing of in vitro Methods

It is imperative to understand the mechanisms of the sensitization (induction) phase of contact hypersensitivity (Vandebriel et al., 2005)

**Induction phase**

- **Structure alert**
- **Skin penetration (Bioavailability)**
- **Protein binding**
- **LC activation**
- **T-cell proliferation**

New in vitro method

- **Cell:** THP-1 cells (human monocyctic leukemia cell line)
- **Markers:** CD86 and CD54

based on Jowsey et al., 2006 J Appl Toxicol, 26, 341-350

LC: Langerhans cells

Lymph node
Human Cell Line Activation Test (h-CLAT)*

**Procedure**

- **THP-1** 1x10^6 cells /mL
- Culture with chemicals, 8 doses based on CV75
- 24h
- Flow cytometric analysis
  - Cell staining (CD86 & CD54)
  - FcR blocking

**Relative Fluorescence Intensity (RFI)**

\[
RFI = \frac{MFI_{chemical treated cells} - MFI_{isotype control cells}}{MFI_{vehicle control cells} - MFI_{vehicle isotype control cells}} \times 100
\]

- MFI = geometric mean fluorescence intensity

**Prediction Model**

- Viability ≥ 50% by Propidium Iodide
- Positive criteria: CD86 RFI ≥ 150% and/or CD54 RFI ≥ 200%
- Positive: 2 of 3 independent data at any dose should exceed the positive criteria

*: Ashikaga et al., 2006 Toxicol In Vitro 767-73., Sakaguchi et al., 2006 Toxicol In Vitro 774-84.
DNCB and Ni (typical allergens) enhanced both CD86 and CD54 expressions but SLS (non-allergen) did not.

Miyazawa et al., Toxicology in Vitro 2007
Today’s presentation

• **Predictive capacity**
  • Evaluation of 117 chemicals by the h-CLAT to compare with LLNA

• **Applicability domain**
  • Applicability domain based on the data base

• **Classification of skin sensitization potency**
  • Using EC150 and EC200 values as the indicator

• **Inter-laboratory study**
  • Ring Trials in the COLIPA (5 labs) and Japan (7 labs)
### Results of 117 Test Chemicals

| Chemical | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wistar | RULM | Human Rats | RULM | Wist
### Comparative evaluation with LLNA and human

#### h-CLAT vs LLNA

<table>
<thead>
<tr>
<th></th>
<th>h-CLAT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+(85)</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>LLNA</td>
<td>-(32)</td>
<td>8</td>
<td>24</td>
</tr>
</tbody>
</table>

- **Sensitivity:** 75/85 (88%)
- **Specificity:** 24/32 (75%)
- **Positive predictivity:** 75/83 (90%)
- **Negative predictivity:** 24/34 (71%)
- **Accuracy:** 99/117 (85%)  

#### h-CLAT vs human

<table>
<thead>
<tr>
<th></th>
<th>h-CLAT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+(51)</td>
<td>46</td>
<td>9</td>
</tr>
<tr>
<td>Human</td>
<td>-(16)</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

- **Sensitivity:** 46/55 (84%)
- **Specificity:** 11/16 (69%)
- **Positive predictivity:** 44/51 (88%)
- **Negative predictivity:** 11/20 (55%)
- **Accuracy:** 57/71 (80%)

**Good predictive capacity, but some false negative / positive**

Nukada et al., WC7 2009, Ashikaga et al., ATLA 2010, Nukada et al., ESCD 2010
<table>
<thead>
<tr>
<th>False negative (1) : Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Applying dose based on cytotoxicity</strong></td>
</tr>
<tr>
<td>Hexyl cinnamic aldehyde</td>
</tr>
<tr>
<td><img src="image" alt="Hexyl cinnamic aldehyde" /></td>
</tr>
<tr>
<td>Abietic acid</td>
</tr>
<tr>
<td><img src="image" alt="Abietic acid" /></td>
</tr>
<tr>
<td>Phthalic anhydride</td>
</tr>
<tr>
<td><img src="image" alt="Phthalic anhydride" /></td>
</tr>
</tbody>
</table>

**The chemical with poor water solubility is one of limitation**

Ashikaga et al., ATLA 2010

*: Calculated with "Water frag" software.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoyl peroxide</td>
<td>Metabolic activity can change the structure</td>
</tr>
<tr>
<td>Geraniol</td>
<td>Metabolic activity or air oxidation can change the structure</td>
</tr>
<tr>
<td></td>
<td>(Basketter et al., Contact Dermatitis, 47(3), 161-164, 2002).</td>
</tr>
<tr>
<td></td>
<td>Oxidation products of geraniol (Geraniol and Neral) augmented CD54 expression</td>
</tr>
<tr>
<td></td>
<td>(Kosaka et al., SOT 2008).</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>Oxidation involves sensitising potential</td>
</tr>
<tr>
<td></td>
<td>(Bertrand et al., Chem Res Toxicol., 10(3), 335-343, 1997).</td>
</tr>
<tr>
<td>Abietic acid</td>
<td>Air oxidation involves expression of sensitizing potential</td>
</tr>
<tr>
<td></td>
<td>(Basketter et al., Food Chem Toxicol 33, 1051-1056, 1995).</td>
</tr>
</tbody>
</table>

The h-CLAT had limitation for some pro- and pre-hapten
Several weak sensitizers could not enhance CD86/CD54 expression

Ashikaga et al., ATLA 2010

<table>
<thead>
<tr>
<th>Weak sensitizers by LLNA classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Bromohexane</td>
</tr>
<tr>
<td><img src="image1.png" alt="1-Bromohexane structure" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LLNA class</th>
<th>Number of tested chemicals</th>
<th>Number of false negatives</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme</td>
<td>8</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Strong</td>
<td>16</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>Moderate</td>
<td>24</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>Weak</td>
<td>23</td>
<td>5</td>
<td>78</td>
</tr>
</tbody>
</table>
EC150 / 200 (Estimated concentration of RFI 150 / 200)

The intermediate value of three experiments was defined as EC150 or EC200.

Calculated based on the calculational procedure of LLNA EC3
Minimum Induction Threshold of h-CLAT – MIT (h-CLAT) -
determined as a smaller value of either EC150 or EC200

Significant correlation with LLNA EC3
Might be useful to classify...

**LLNA EC3 ...?**
**Proposed GHS subcategories ...?**

Ref. Proposed GHS subcategories for skin sensitization based on LLNA EC3 and the example of prediction

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Animal test results (using LLNA data)</th>
<th>Cut off (h-CLAT)</th>
<th>Accuracy(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A (Strong sensitizer)</td>
<td>EC3 ≤ 2%</td>
<td>MIT 10 μg/mL</td>
<td>78.8</td>
</tr>
<tr>
<td>1B (Weak sensitizer)</td>
<td>EC3 &gt; 2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
COLIPA and Japanese Ring Trials

**Purpose**
- Protocol transferability
- Inter-laboratory reproducibility
- Predictive capacity

**Goals**
- Identify unexpected problems with either test design or procedures
  - Protocol optimization/standardization
- Identify problems with data analysis / interpretation
  - Prediction model refinement

**Members**
- **COLIPA:** P&G, L’Orel, Henkel-Phnion, Shiseido and Kao
- **Japan:** Kanebo Cosmetics, Kose, Lion, Nippon Menard Cosmetic, Pola Chemical Industries, Shiseido and Kao
## COLIPA 4th Ring Trial summary data

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Potency</th>
<th>Lab B</th>
<th>Lab C</th>
<th>Lab D</th>
<th>Lab E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPD</td>
<td>Strong</td>
<td>+ (2/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
</tr>
<tr>
<td>Methyldibromo glutaronitrile</td>
<td>Strong</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (2/3)</td>
<td>+ (3/3)</td>
</tr>
<tr>
<td>2-Mercaptobenzothiazole</td>
<td>Strong</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
</tr>
<tr>
<td>Cinnamic Aldehyde</td>
<td>Moderate</td>
<td>- (1/3)</td>
<td>+ (3/3)</td>
<td>+ (2/3)</td>
<td>+ (3/3)</td>
</tr>
<tr>
<td>Tetramethylthiuram Disulfide</td>
<td>Moderate</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>NS</td>
<td>- (0/3)</td>
<td>- (0/3)</td>
<td>- (0/3)</td>
<td>- (0/3)</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>NS</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (2/3)</td>
<td>+ (3/3)</td>
</tr>
</tbody>
</table>

7 test chemicals (5 allergens, 2 non-allergens), 4 labs
- Cinnamic Aldehyde: one false negative data
- Salicylic acid: false positive in all labs
- Good inter-laboratory reproducibility
- Almost good predict performance

Sakaguchi et al., Toxicology in Vitro 2010
Japanese 1st Ring Trial

- 3 test chemicals (2 allergens, 1 non-allergen), 7 labs
- Test doses were same in all labs

Good inter-lab reproducibility
Good predict performance

Ashikaga et al., AATEX 2008
Summary

• **Predictive capacity (117 chemicals)**
  - Good prediction performance (accuracy: 85%/80% between the h-CLAT/human and LLNA) was observed.

• **Applicability domain**
  - Possible applicability domain was solubility, metabolic activity, sensitivity, etc.

• **Classification of skin sensitization potency**
  - MIT might be useful to predict the allergic potency of chemicals classified by GHS classification

• **Inter-laboratory study**
  - COLIPA: 15 chemicals, approx 85% predicted correctly
  - Japan: 8 chemicals, approx 96% predicted correctly
  - Good inter-lab reproducibility and predictive performance
ECVAM prevalidation study

• **Liaison:**
  - JaCVAM and ICCVAM

• **Test methods:**
  - Direct Peptide Reactivity Assay (DPRA)
  - Myeloid U937 Skin Sensitization Test (MUSST)
  - human Cell Line Activation Test (h-CLAT)

• **Main purpose**
  - The assessment of the robustness and reliability

• **Experimental design**
  - 24 coded chemicals in three (or four) laboratories each for the assessment of the within- and between-laboratory reproducibility
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