Skin sensitisation: Now and the Future
43rd ICGM - New Orleans - 17th March 2016
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Scientist
donna.macmillan@lhasalimited.org
Overview

• Derek Nexus and skin sensitisation
• Improvements and new features
  • Alerts and performance
  • EC3 potency predictions
• Future features/developments
  • Negative predictions for skin sensitisation
  • Integrated Testing Strategies (ITS)
• The importance of data sharing
• Questions
Derek Nexus and skin sensitisation
What is Skin Sensitisation?

- Skin sensitisation is the process of a chemical causing an allergic reaction leading to allergic contact dermatitis (ACD).

- Develops in two stages:

  (1) Induction:
  chemical (hapten) forms a stable conjugate (hapten-protein complex) with carrier proteins within the skin initiating a cascade which ends with proliferation of allergen specific T-cells.

  (2) Elicitation:
  subsequent contact with the same allergen leads to the hapten-protein complex triggering the allergen specific T-cells which induces inflammatory cytokines and ACD.

- Has a well-defined Adverse Outcome Pathway (AOP) (OECD 2012)

Skin Sensitisation AOP

- An Adverse Outcome Pathway (AOP) is the sequence of events leading to an *in vivo* outcome of interest - skin sensitisation elicited by covalent binding of chemicals to skin proteins.
- Published by the OECD in 2012.

- **4 key events:**
  - MIE - covalent binding of hapten to skin protein
  - 2nd and 3rd key events - cellular response by keratinocytes and dendritic cells
  - 4th key event - organ response - activation of T-cells
  - Adverse Outcome - skin sensitisation

Non-animal alternatives

• Implementation of EU Regulation 1223/2009 (Cosmetics Regulation) has led to increased interest in non-animal alternatives.

• DPRA, KeratinoSens and h-CLAT have been validated by the OECD.

• Generally agreed that more than one non-animal assay will be required to replace the current ‘gold standard’, the LLNA.
  • Focus has turned to combining assays in an integrated testing strategy (ITS).
  • ITS using *in silico* models may be a good way to predict skin sensitisation.
New and future functionality in Derek Nexus

QUERY COMPOUND → SS alert?

- **YES**
  - Hazard prediction
  - Potency prediction
  - In vitro data ITS
    - Likelihood
    - EC3%

- **NO**
  - Negative predictions
    - Non-sensitiser

**Existing functionality**

**Future functionality**
Improvements to alerts
Improved alerts - performance

- Skin sensitisation is a key endpoint in DX.
  - Ongoing development work on this endpoint at Lhasa.
- Currently 88 skin sensitisation alerts.
- Performance against public data is good:

<table>
<thead>
<tr>
<th></th>
<th>Acc</th>
<th>Sens</th>
<th>Spec</th>
<th>PP</th>
<th>NP</th>
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<tr>
<td>Derek 2015</td>
<td>74</td>
<td>78</td>
<td>70</td>
<td>73</td>
<td>76</td>
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<tr>
<td>Derek 2014</td>
<td>72</td>
<td>72</td>
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<td>72</td>
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</tbody>
</table>

*Analysis based on a data set of 1316 sensitisers and 1283 non-sensitisers based on conservative combination of results from the LLNA and/or guinea pig assays.

Acc = (TP+TN)/<all compounds>
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Improved alerts - performance

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<table>
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<tr>
<th>Knowledge base</th>
<th>Acc</th>
<th>Sens</th>
<th>Spec</th>
<th>PP</th>
<th>NP</th>
<th>Alerts</th>
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<tr>
<td>Derek 2015</td>
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Spec = TN/<all sensitises>
PP = TP/<TP+FP>
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Improved alerts - summary sentence
Skin sensitisation: murine local lymph node assay (LLNA), guinea pig maximisation test (GPMT)

Potential mechanism: Hapten acting as an electrophilic acylating agent [Aptula and Roberts]
Potency (EC3) predictions
New feature: Potency prediction

• Various *in silico* models can predict **hazard** (sensitiser/non-sensitiser).

• Very few predict **risk**.

• **Risk** can be assessed using EC3 (LLNA).
  • Extreme
  • Strong
  • Moderate
  • Weak
  • Non-sensitiser

• Risk prediction would be of significant value to cosmetics and other industries interested in making risk assessments.
New feature: Potency prediction - workflow

1. **Query Compound**
   - **Fires DX alert?**
     - **YES**
       - **Exact match?**
         - **YES**
           - **Experimental EC3**
         - **NO**
           - **Over 3 NN (up to 10)**
             - **Predicted EC3**
     - **NO**
       - **Similarity score calculated for NN activating the same alert**
         - **Over 3 NN (up to 10)**
           - **Predicted EC3**
         - **Under 3 NN**
           - **Insufficient data for EC3 prediction**
   - **NO**
     - **No EC3 prediction**

*NN = Nearest Neighbours (structurally similar compounds firing the same alert)*
New feature: Potency prediction - example
Negative predictions
Future feature: Negative predictions

• Negative predictions are important for regulatory toxicology.
  • Need to be sure of ‘absence of hazard.’
• A methodology for making negative predictions has been successfully implemented for the bacterial, *in vitro* mutagenicity endpoint in Derek:
  • Based on a structural-feature based search of a reference data set for non-alerting compounds.
• Methodology trialled for skin sensitisation endpoint:
  • Produced robust, repeatable results.
  • Currently being implemented into Derek Nexus.
Integrated Testing Strategies (ITS)
ITS

• Alternative approaches generally model one of the key events in the AOP.

• Generally accepted that a combination of *in vitro*, *in chemico* and *in silico* methods will be required to replace *in vivo* tests.

• Not clear which key events determine allergen potency.

• Considerable progress has been made in ITS which predict hazard.
Assessing skin sensitization hazard in mice and men using non-animal test methods

Daniel Urbisch a, Annette Mehling b, Katharina Guth a, Tzutzuy Ramirez a, Naveed Honarvar a, Susanne Kolle a, Robert Landsiedel a,b, Joanna Jaworska c, Petra S. Kern d, Frank Gerberick c, Andreas Natsch f, Roger Emter f, Taka Ashikaga g, Masaaki Miyazawa h, Hitoshi Sakaguchi h

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b BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany
c Procter & Gamble NV, 100 Temsebaan, 1853 Strombeek-Bever, Belgium
d Procter & Gamble Technology (Beijing) Co., Ltd., China
e Procter & Gamble Company, Cincinnati, OH, USA
f Givaudan Schweiz AG, Ueberlandstrasse 138, CH-8600 Diébensdorf, Switzerland
g Shibudo Research Center, Shibudo Co., Ltd., 2-2-1 Hayabuchi, Tsuzuki-ku, Yokohama-shi, Kanagawa 224-8558, Japan
h Safety Science Research Laboratories, Kao Corporation, 2606 Akabane, Ichikai, Haga, Tochigi 321-349, Japan

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<td>0.79</td>
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ITS using DX and 2 in vitro assays

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![Table with data]

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<td>0.80</td>
</tr>
<tr>
<td>Lhasa DT</td>
<td>0.85</td>
<td>0.86</td>
<td>0.81</td>
<td>0.75</td>
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</table>
Predicting skin sensitisation using a decision tree integrated testing strategy with an *in silico* model and *in chemico/in vitro* assays

Donna S. Macmillan*, Steven J. Canipa, Martyn L. Chilton, Richard V. Williams, Christopher G. Barber

*Lhasa Limited, Granary Wharf House, 2 Canal Wharf, Leeds, LS11 5JS, UK*

<table>
<thead>
<tr>
<th>DX result</th>
<th>In vitro domain</th>
<th>1st in vitro result</th>
<th>2nd in vitro result</th>
<th>Overall call</th>
<th>Predictivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alert fired</td>
<td>Within domain: 77 90% PP</td>
<td>Positive: 23-63</td>
<td>Positive: 0-12</td>
<td>sensitizer</td>
<td>96% PP</td>
</tr>
<tr>
<td></td>
<td>Outside of domain: 74 81% PP</td>
<td>Negative: 5-14 44% NP</td>
<td>Negative: 1-8</td>
<td>non-sensitizer</td>
<td>77% PP</td>
</tr>
<tr>
<td>No alert fired</td>
<td>Within domain: 43 72% NP</td>
<td>Positive: 8-17 48% PP</td>
<td>Positive: 3-8</td>
<td>sensitizer</td>
<td>58% NP</td>
</tr>
<tr>
<td></td>
<td>Outside of domain: 19</td>
<td>Negative: 13-27</td>
<td>Negative: 0-9</td>
<td>non-sensitizer</td>
<td>81% PP</td>
</tr>
</tbody>
</table>

**Highlights**

- 213 test compounds with LLNA, human, DPRA, KeratinoSens, LuSens, h-CLAT and U-SENS results were used to develop an ITS.
- The ITS decision tree used Derek Nexus and a maximum of two *in chemico/in vitro* assays to evaluate skin sensitisation.
- Fewer *in chemico/in vitro* assays required when using the decision tree (128) instead of a non- *in silico* based ITS (426-639).
- The (mean) performance was as follows: accuracy 85%, positive predictivity 86%, negative predictivity 81% and coverage 75%.
Benefits of *in vitro +* Derek Nexus over 2/3 WoE and LLNA

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Combination of assays</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LLNA</td>
<td>In vitro (2/3 WoE)</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>£££ (~ £5.5k)</td>
<td>£££ (~ £5k)</td>
</tr>
<tr>
<td><strong>No of animals</strong></td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td><strong>Timescale</strong></td>
<td>Weeks</td>
<td>Days</td>
</tr>
<tr>
<td><strong>No of compounds</strong></td>
<td>1 per assay</td>
<td>1 per assay</td>
</tr>
<tr>
<td><strong>No of assays</strong></td>
<td>1</td>
<td>2-3 (426-639)</td>
</tr>
</tbody>
</table>

**Predictions**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Metals?</strong></td>
<td>Not often</td>
<td>Poorly</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Pre-/pro-haptens</strong></td>
<td>Some</td>
<td>Not often</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Lipophilic compounds</strong></td>
<td>Yes</td>
<td>Poorly</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The importance of data-sharing
Why is data sharing important?

• Encourages collaboration which benefits the scientific community.

• Gaps in the chemical space covered by *in silico* models can exist.
  • Derek Nexus alerts are built mainly on public data.

• By donating proprietary data, these gaps can be filled.
  • Model chemical space unique to each member.
  • Can improve predictivity in the chemical space most important to members.
  • Generalise models for mutual benefit.

• The following slides show a **case study** using LLNA data donated by one of our members…
Data sharing - coverage

• Case study - coverage of chemical space

• **Conclusion:** Proprietary data covers chemical space not already covered by public data.
**Member data set - Performance**

- 467 compounds
- Mainly negative results
  - Bias = 76% negative
- 74 FP
- 62 FN

<table>
<thead>
<tr>
<th>Skin</th>
<th>Metrics (%)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Data set</td>
<td>Se</td>
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<tr>
<td></td>
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<td>77</td>
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<tr>
<td></td>
<td>Member</td>
<td>44</td>
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</tbody>
</table>
Member data set – Alert summary

• 6 new alerts

• 5 modifications to existing alerts

• 5 potential new alerts/alert modifications require more data
Results - Member data

Performance Metric

<table>
<thead>
<tr>
<th>Metric</th>
<th>Derek 2014</th>
<th>After data sharing</th>
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<tbody>
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<td>Acc</td>
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<td>78</td>
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<td>Se</td>
<td>44</td>
<td>66</td>
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<tr>
<td>Sp</td>
<td>79</td>
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<td>PP</td>
<td>40</td>
<td>53</td>
</tr>
<tr>
<td>NP</td>
<td>82</td>
<td>89</td>
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%
Results - Public data

<table>
<thead>
<tr>
<th>Performance Metric</th>
<th>Derek 2014</th>
<th>After data sharing</th>
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<tbody>
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<td>Acc</td>
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<td>74</td>
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<tr>
<td>Se</td>
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<tr>
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<td>73</td>
</tr>
<tr>
<td>NP</td>
<td>72</td>
<td>75</td>
</tr>
</tbody>
</table>
Data sharing - overview

- Benefits - Improves predictivity of both public and proprietary data sets.
- Benefits - Can result in collaborative publication.
- Especially interested in sharing EC3 data.
- Possible skin data consortium in the pipeline.
  - Donate specified number of compounds with EC3.
  - Given access to custom EC3 model in Derek.
  - All consortium data contained within the custom model.
  - Is that something our members would be interested in?
Conclusions

- Improved transparency of skin sensitisation alerts.

- Derek Nexus now makes quantitative (risk) predictions.

- Published on using Derek Nexus as part of an ITS with improved predictivity compared to in vitro assays alone.

- Science has been agreed for negative predictions - currently being implemented.

- Data sharing is the way forward to improve skin sensitisation predictions in Derek Nexus.
Thank you for your attention

Any questions?