


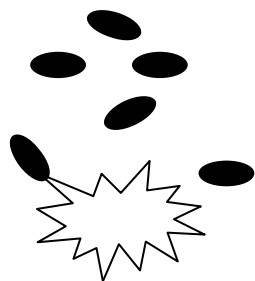
# VITASENS<sup>®</sup>

a cell-based alternative  
to identify skin sensitizers  
by gene expression

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 Forinvitox, May 12, 2009

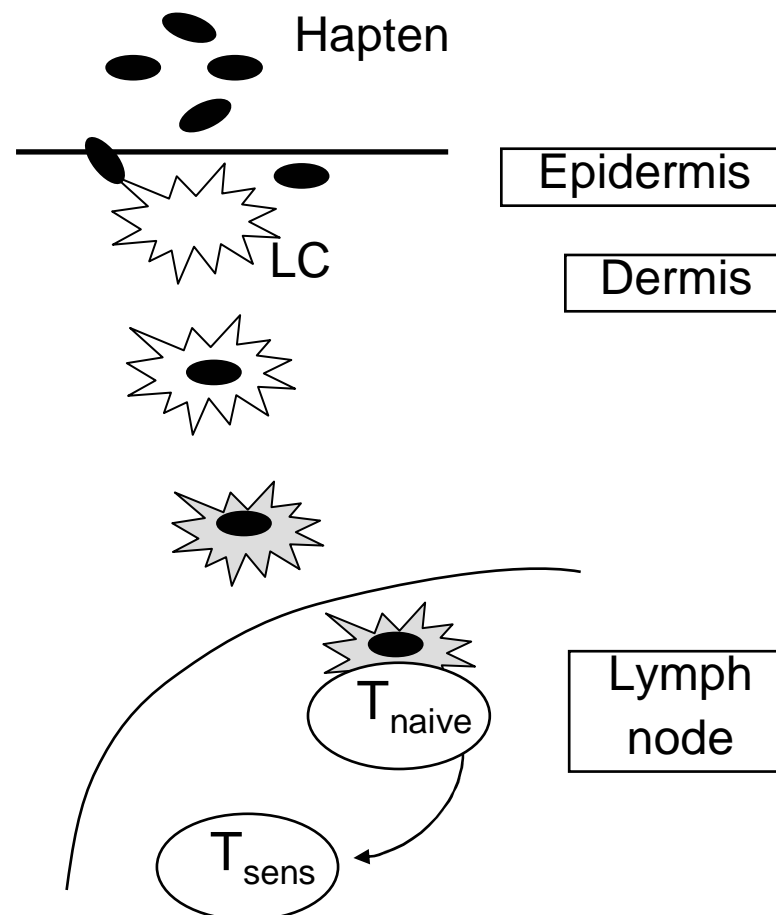
## IN VITRO



### Dendritic cells

primary, human  
CD34<sup>+</sup> progenitor-derived

## IN VIVO



## Past CD34<sup>+</sup>DC research at VITO-CARDAM

- **1998 – 2002** Surface markers and cytokines
- **2002** Gene expression
  - **2002- 2006** Transcriptomics (4 sensitizers, 2 irritants)  
selection of discriminative genes: 13 genes  
*Schoeters E et al., Mol Immunol 44, 2007*
  - **2006** Real-time RT-PCR

### Database: gene expression signatures

(method Real-time RT-PCR )

13 genes (solvent versus exposure)

3 exposure times

3 exposure concentrations

21 chemical compounds (10 sens, 11 N-sens)

3 cell cultures / compound (minimal)

(73 donor samples = 73 cell cultures)

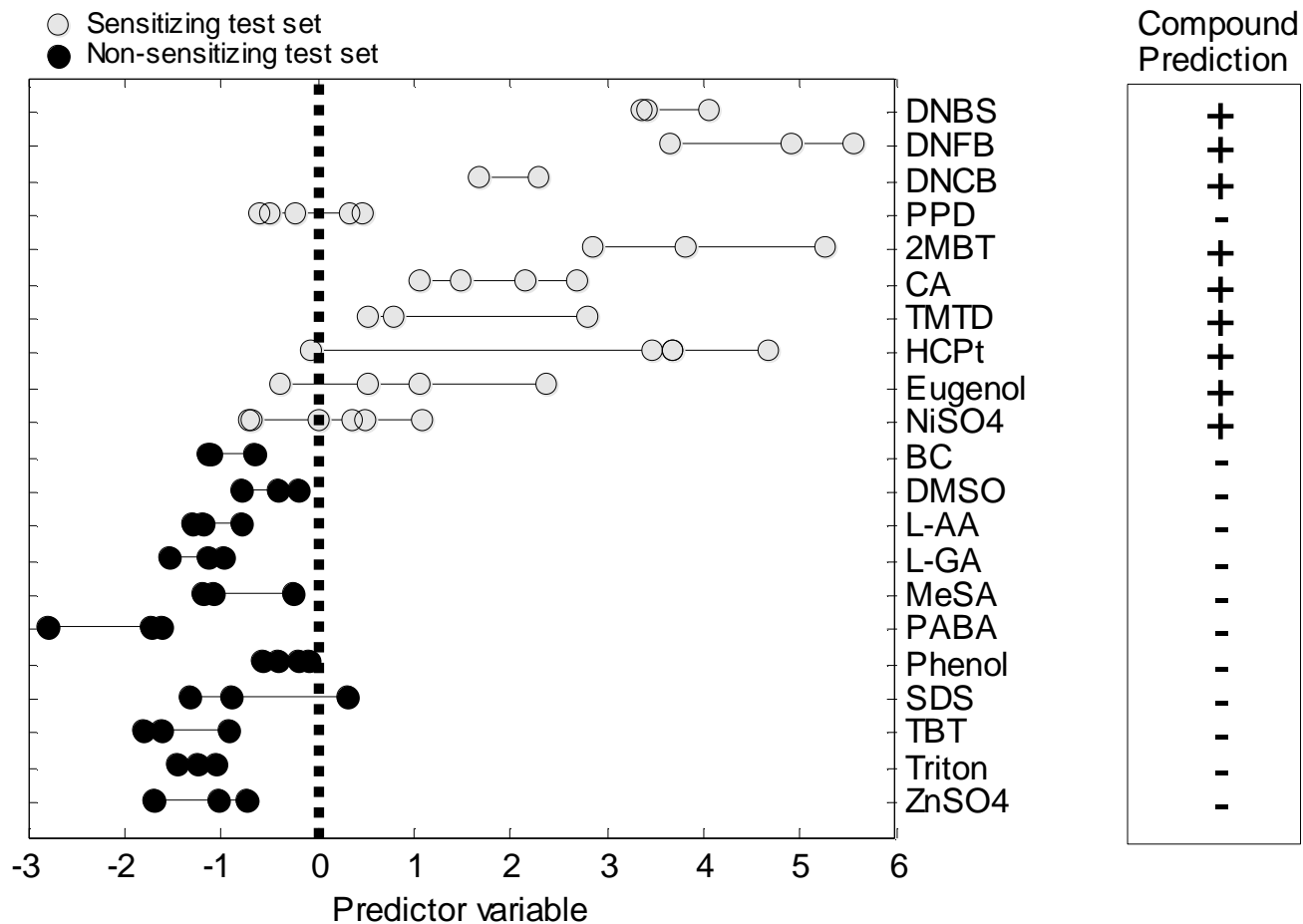
### Sensitizers

### Non-sensitizers

<b>Dinitrobenzenesulfonic acid</b>	<b>DNBS</b>	<b>Benzalkonium Chloride</b>	<b>BC</b>
<b>Dinitrofluorobenzene</b>	<b>DNFB</b>	<b>Dimethylsulfoxide</b>	<b>DMSO</b>
<b>Dinitrochlorobenzene</b>	<b>DNCB</b>	<b>L-Ascorbic Acid</b>	<b>L-AA</b>
<b>p-phenylenediamine</b>	<b>PPD</b>	<b>L-Glutamic Acid</b>	<b>L-GA</b>
<b>2-mercaptobenzothiazole</b>	<b>2MBT</b>	<b>Methyl salicylate</b>	<b>MeSA</b>
<b>Cinnamaldehyde</b>	<b>CA</b>	<b>p-Aminobenzoic Acid</b>	<b>PABA</b>
<b>Tetramethylthiuram disulfide</b>	<b>TMTD</b>	<b>Phenol</b>	<b>phenol</b>
<b>Ammonium hexachloroplatinate IV</b>	<b>HCPt</b>	<b>Sodium Lauryl/Dodecyl Sulphate</b>	<b>SDS</b>
<b>Eugenol</b>	<b>eugenol</b>	<b>Tributyltin Chloride</b>	<b>TBT</b>
<b>Nickel Sulfate</b>	<b>NiSO<sub>4</sub></b>	<b>Triton X-100</b>	<b>triton</b>
		<b>Zinc sulphate</b>	<b>ZnSO<sub>4</sub></b>

### Statistical construction

- Reduction of inputs → 1 conc., 2 genes: CREM & CCR2
- Discriminant analysis:  
predictor variable = weighted average of gene expressions
- Cross-validation:  
Each of 21 chemicals tested as independent test compound



<b>Sensitivity</b> 9/10	<b>90%</b>
<b>Specificity</b> 11/11	<b>100%</b>
<b>Concordance</b> 20/21	<b>95%</b>

*Hooyberghs J et al., Toxicol. Appl. Pharmacol. 231, 2008*

- VITASENS<sup>®</sup> gene markers: primary DC versus THP-1 cell line

*Lambrechts N, Verstraelen S et al., Toxicol. Appl. Pharmacol. 236, 2009*

- Metabolization in DC

Eugenol, p-phenylenediamine (PPD), Bandrowski's base (BB)

- Potency: sensitization classes

Extra compounds

- Pathway analysis

### VITOSENS® is operational

- Test definition
- Within laboratory variability
- Ethical concerns (primary material)

### TO DO

- Transferability
- Between lab variability
- Predictive capacity
- ...

Patent pending: WO2008037806

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