A cell-based *in vitro* alternative to identify skin sensitizers by gene expression

Analysis of a statistical prediction model: VITOSENS®

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Allergic contact dermatitis (ACD):

An immunologically mediated cutaneous reaction to a substance.

Type IV allergic reaction (delayed hypersensitivity), sensitization of T-lymphocytes

Health issue

ACD affects 7% entire population

Eczema & CD: 85% of all occupational skin diseases in the working population

EU Regulations

REACH: toxicological assessment of chemical substances

including sensitizing potential

→ Need for predictive testing

Current validated sensitizations tests

e.g. Guinea-pig tests

Mouse local lymph node assay

7th amendment of cosmetics directive 76/768/EEC

Ban on animal testing

→ Need for alternative tests for skin sensitization

Induction phase

Chemical (hapten) penetrates skin and reacts with protein(s)

Chemical-protein complex recognised by Langerhans cells (LC).

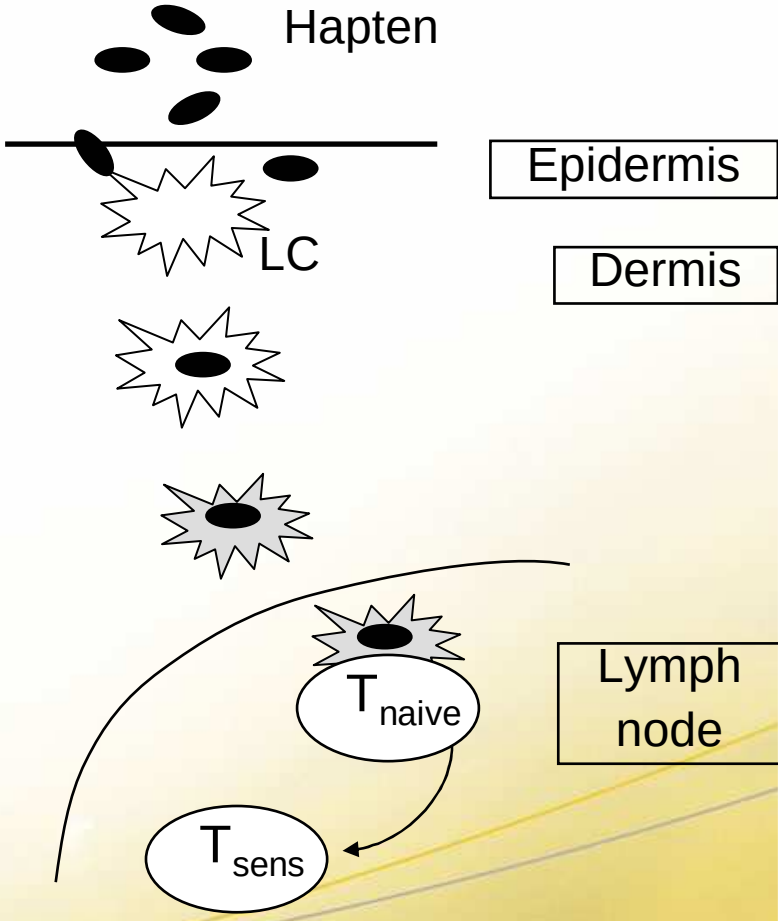
LC migration from skin to drainingn lymph node.

LC maturation.
Enhanced expression of costimulatory molecules.

Mature LC presents chemical to T cells

Proliferation of specific T cells

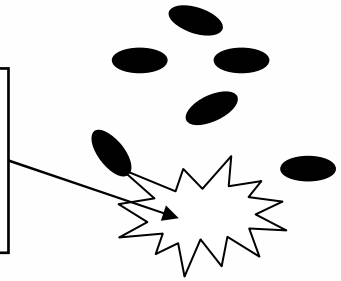
Chemical-specific T-cells released into the systemic circulation



IN VITRO

VITO strategy

Human CD34+ progenitor-derived dendritic cells



Research including succession of three PhD's

1998 -2002

Surface markers and cytokines

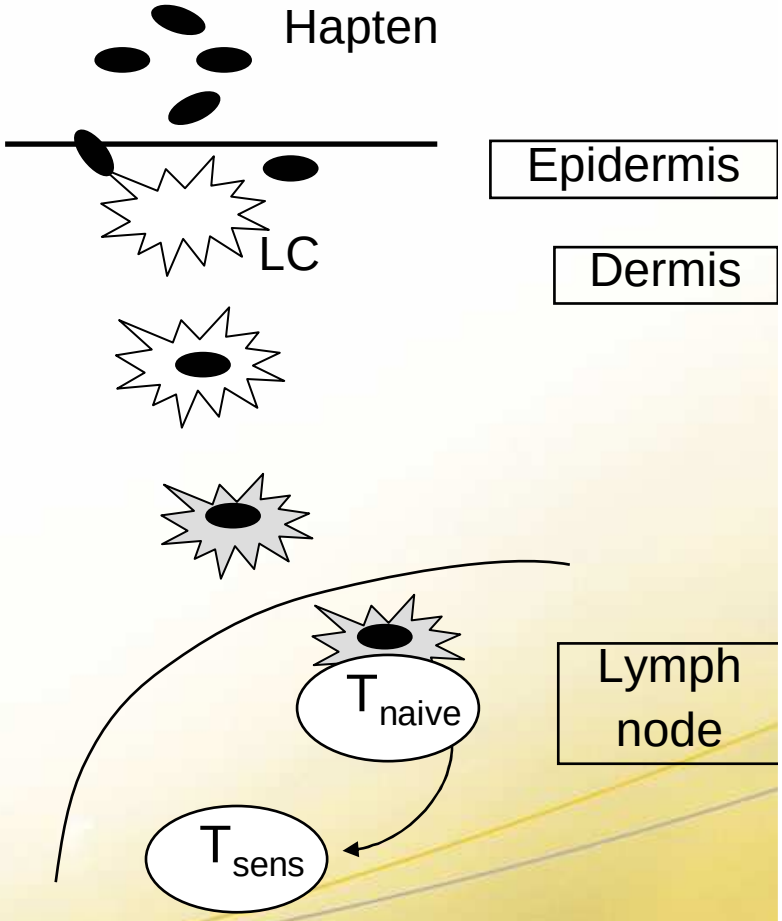
2002 – 2006

Transcriptomics, selection of discriminative genes

2006 – current

Real-time RT-PCR: prediction model
pathway analysis (Poster I-SUP)

IN VIVO



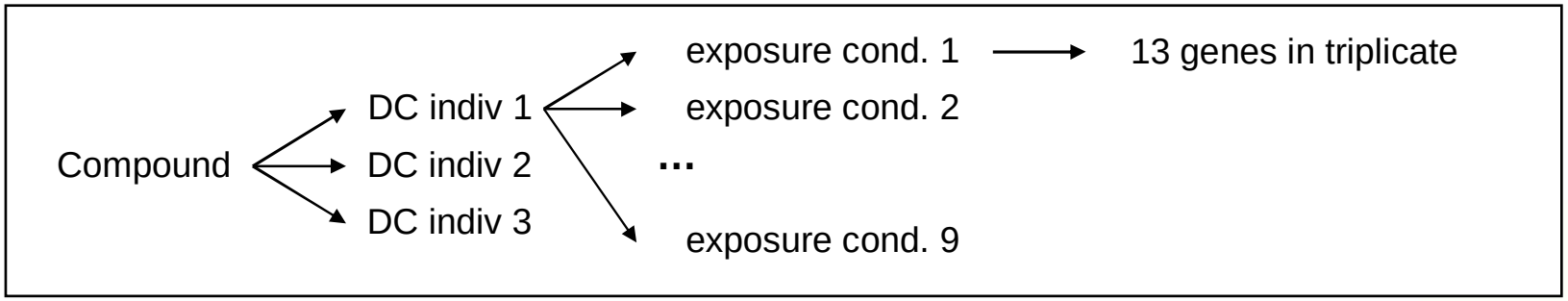
13 genes

ABCA6	ATP-binding cassette, sub-family A (ABC1), member 6
AQP3	Aquaporin 3
CCR2	Chemokine (C-C motif) receptor 2
CCR7	Chemokine (C-C motif) receptor 7
CREM	CAMP responsive element modulator
CXCR4	Chemokine (C-X-C motif) receptor 4
ENC	Ectodermal-neural cortex (with BTB-like domain)
MAD	MAX dimerization protein 1
NINJ	Ninjurin 1
PBEF1	Pre-B-cell colony-enhancing factor 1
PSCDBP	Pleckstrin homology, Sec7 and coiled-coil domains, binding protein
PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
SLC2A3	Solute carrier family 2 (facilitated glucose transporter), member 3

21 chemical compounds

Sensitizers		non-sensitizers	
Dinitrobenzenesulfonic acid	**DNBS**	**Benzalkonium Chloride**	**BC**
Dinitrofluorobenzene	**DNFB**	**Dimethylsulfoxide**	**DMSO**
Dinitrochlorobenzene	**DNCB**	**L-Ascorbic Acid**	**L-AA**
p-phenylenediamine	**PPD**	**L-Glutamic Acid**	**L-GA**
2-mercaptobenzothiazole	**2MBT**	**Methyl salicylate**	**MeSA**
Cinnamaldehyde	**CA**	**p-Aminobenzoic Acid**	**PABA**
Tetramethylthiuram disulfide	**TMTD**	**Phenol**	**phenol**
Ammonium hexachloroplatinate IV	**HCPt**	**Sodium Lauryl/Dodecyl Sulphate**	**SDS**
Eugenol	**eugenol**	**Tributyltin Chloride**	**TBT**
Nickel Sulfate	**$NiSO_4$**	**Triton X-100**	**triton**
		Zinc sulphate	**$ZnSO_4$**

Experimental setup



Each chemical used to expose DC of ≥3 donor samples (in total 73 samples)

Each exposure experiment consists of 9 conditions:

time: 6h, 11h, 24h

conc: IC20, IC10, IC5 & solvent control

At each exposure condition: expression fold change of 13 genes in triplicate

exposed versus solvent control

Dimensionality reduction

1. Selection of exposure concentration & exposure time

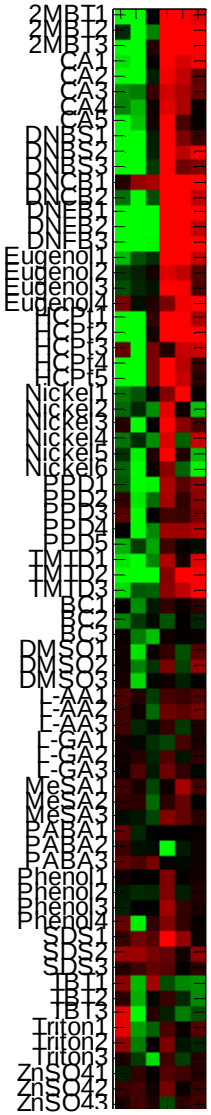
~~IC5~~, ~~IC10~~, IC20 6h, 11h, 24h

2. Selection of genes

CREM	p = 4.8856e-011	} in this presentation
CCR2	p = 7.0736e-011	
SLC2A3	p = 7.4324e-009	
PBEF1	p = 1.2882e-007	
MAD	p = 8.3656e-007	
AQP3	p = 1.0480e-006	
PSCDBP	p = 1.0480e-006	
PTGS2	p = 4.3343e-005	
NINJ	p = 8.5548e-004	
ABCA6	p = 6.4114e-003	
ENC	p = 8.6285e-003	
CXCR4	p = 1.1507e-002	
CCR7	p = 1.1875e-002	

Construction of classifier

heatmap
red: >0 green: <0



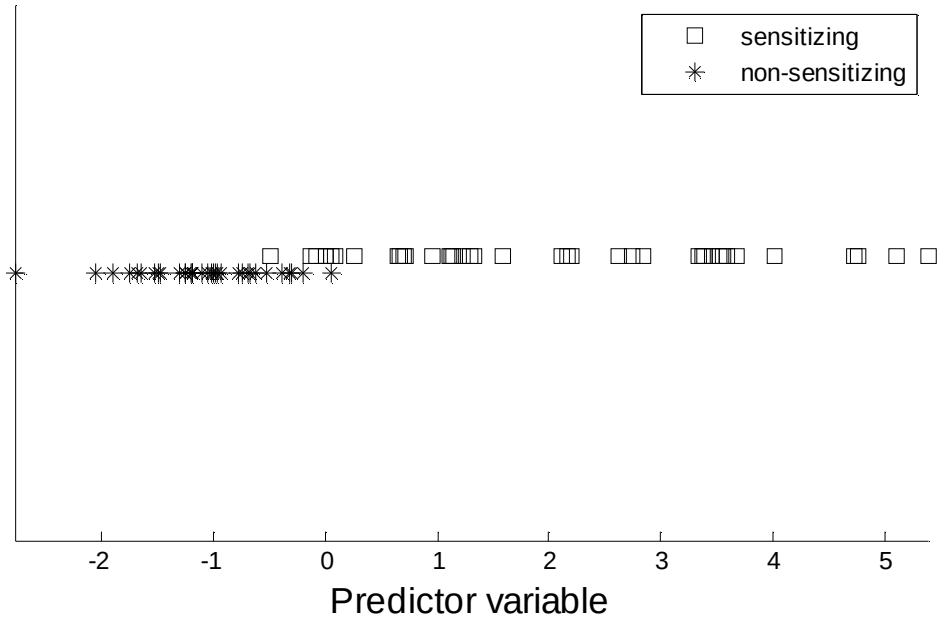
CCR2 (6h, 11h, 24h)
CREM (6h, 11h, 24h)

Time-weighted average

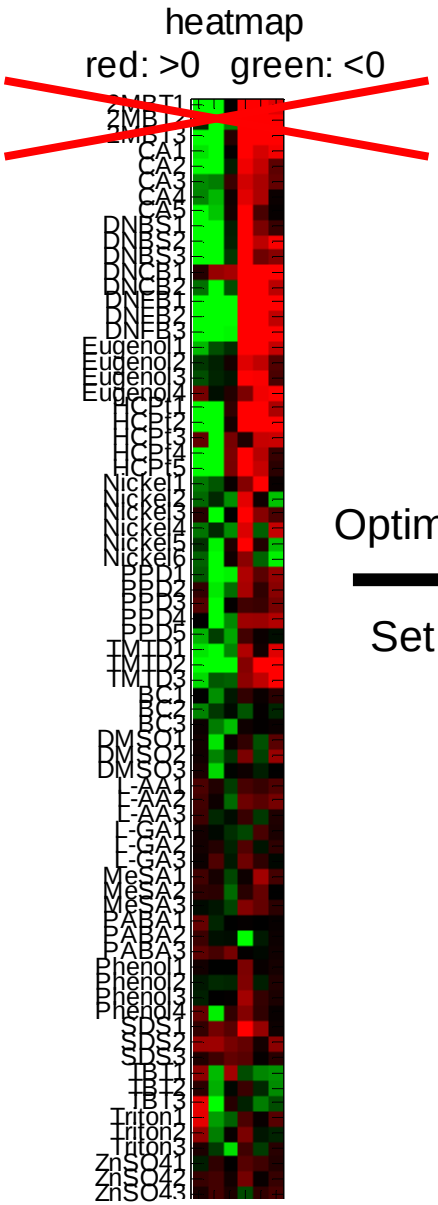
Gene-weighted average

Predictor variable = optimised weighted average

Predictor variable per exposed donor sample



Cross-validation

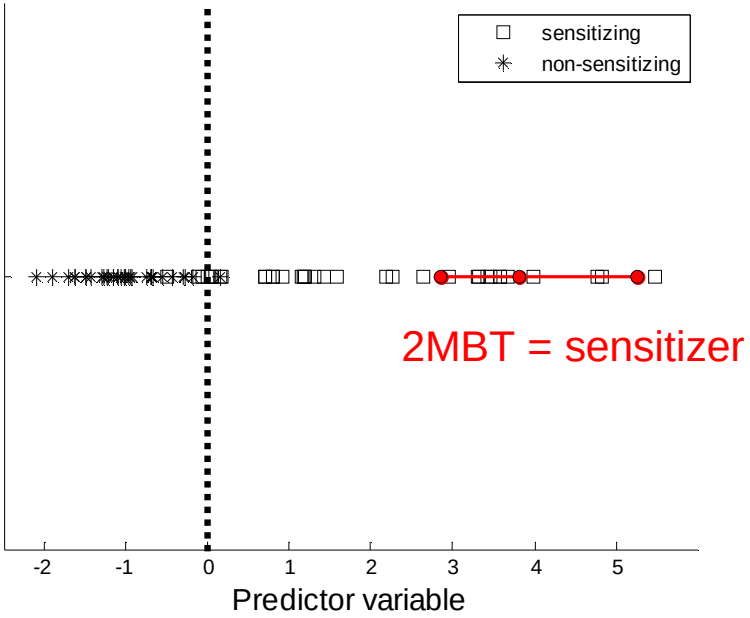


CCR2 (6h, 11h, 24h)

CREM (6h, 11h, 24h)

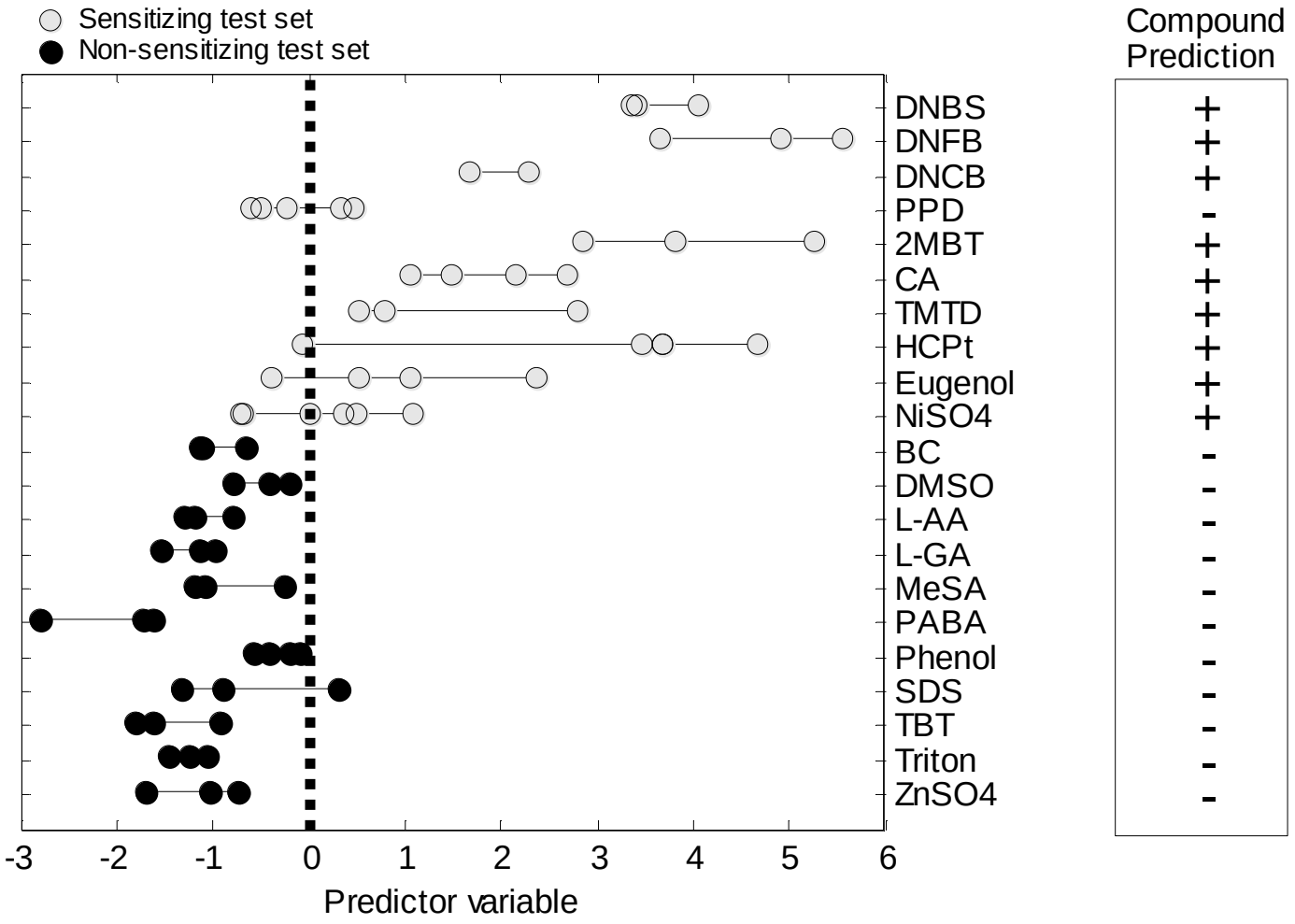
Optimise weights

Set threshold



2MBT = sensitizer

Cross-validation VITOSENS®



Contingency table of the VITOSENS® model

	Predicted sensitizing	Predicted non-sensitizing	Total
Sensitizing	**32**	**7**	39
Non-sensitizing	**1**	**33**	34
Total	33	40	73

Sensitivity = 32/39	**82%**
Specificity = 33/34	**97%**
Concordance = (32+33)/73	**89%**

4 conclusions

Present:	potential as a human *in vitro* alternative in an integrated strategy towards reduction of animal use in skin sensitization hazard assessment
Future:	- further validation - sensitizing potency - biological significance

Refs: - Schoeters E et al. Mol Immunol 2007;44(12):3222-33.
- Hooyberghs J et al. Toxicol. Appl. Pharmacol.
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