Immunotoxicogenomics Workshop

Gene Expression Patterns in Allergic Sensitization in the Skin

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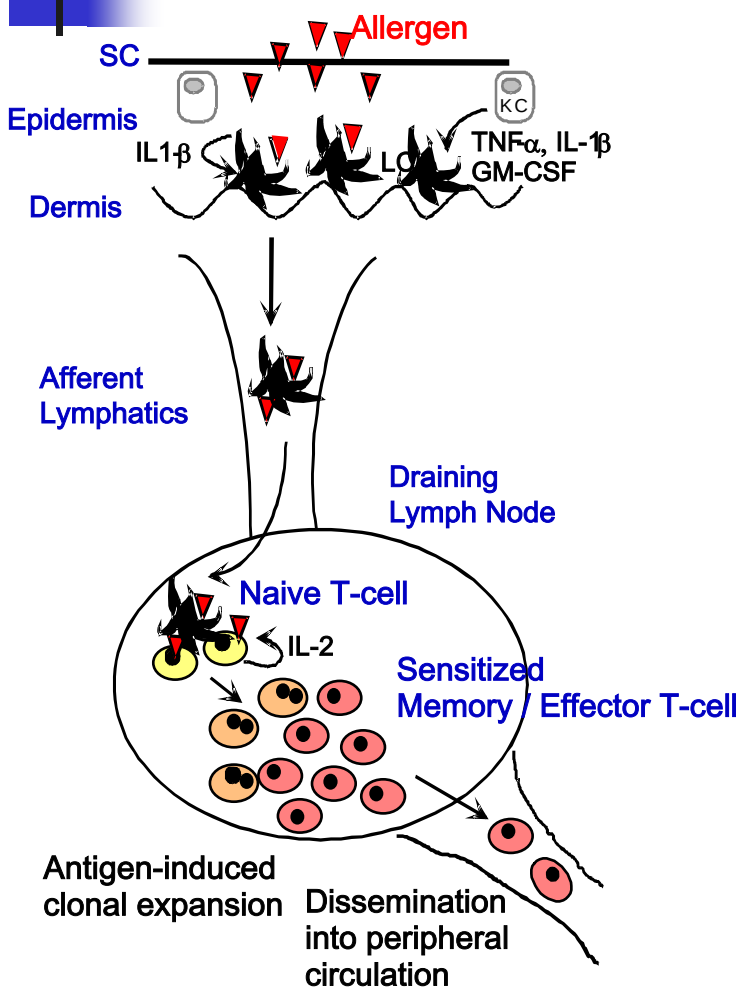
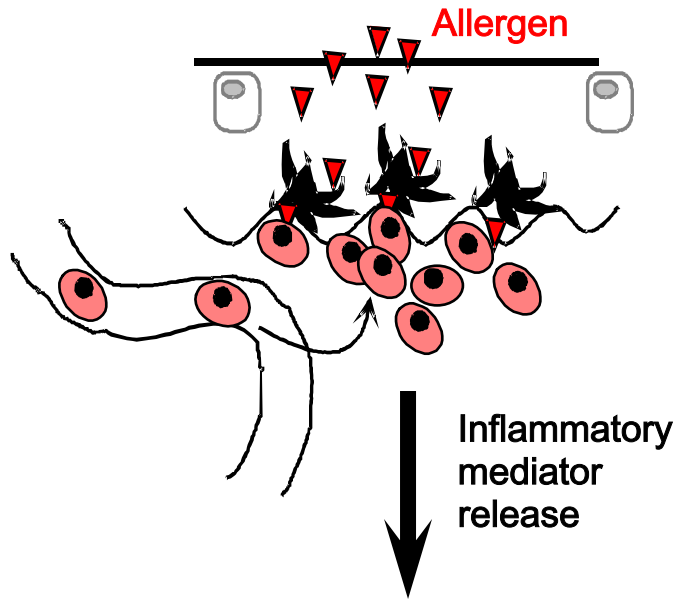
IMMUNOLOGIC MECHANISM OF CONTACT SENSITIZATION

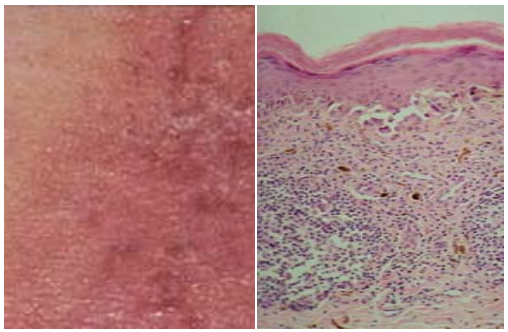
Sensitization Phase



Elicitation Phase



Erythema
Edema
Vesiculation



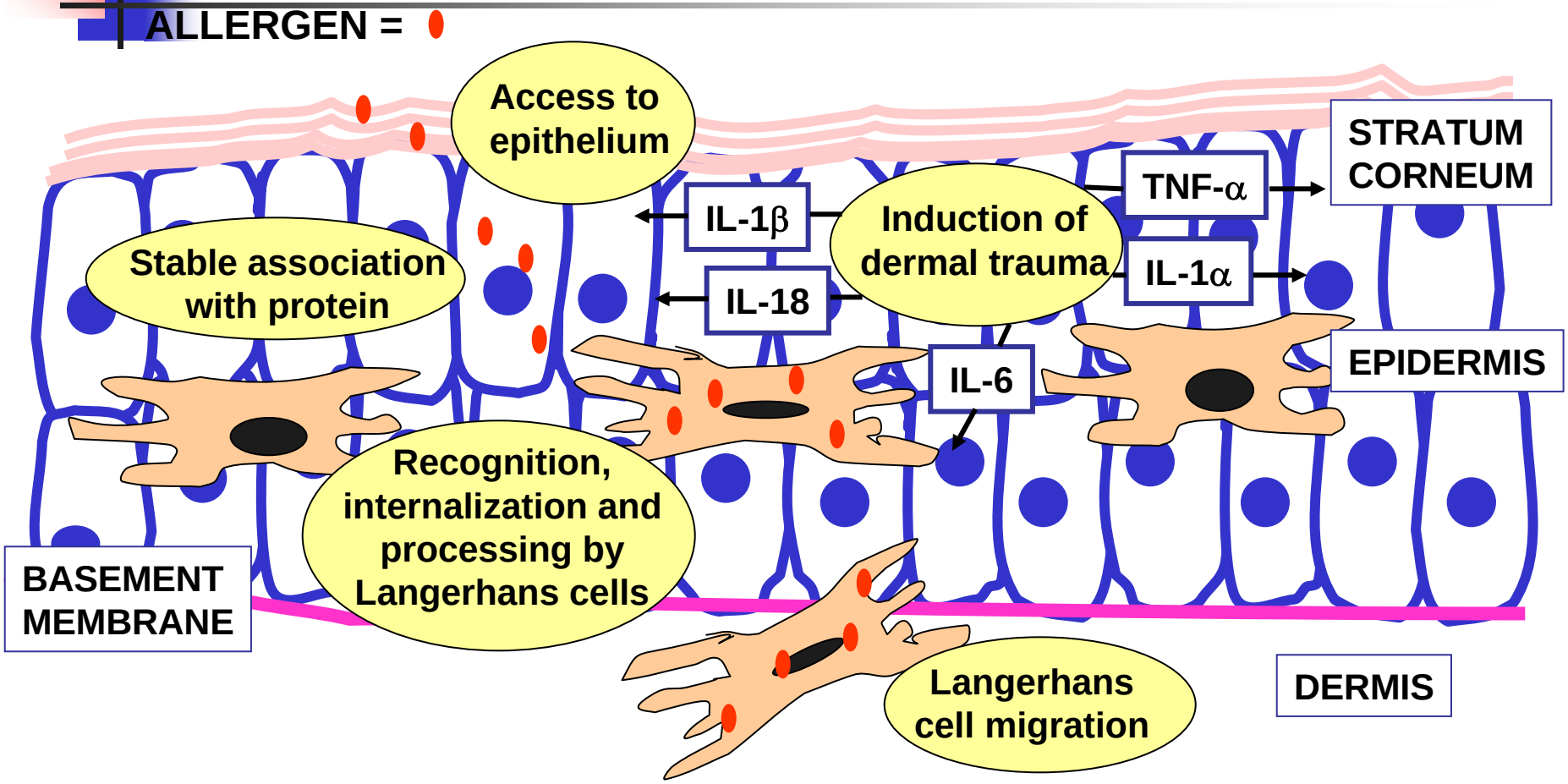
Status of Alternative Approaches and *In Vitro / In Silico* Methods

- ***At the moment validated non-animal approaches do not exist for the identification of skin sensitizers***

- ***However, recent advances in a number of fields have provided the foundation for excellent progress to be made in the coming years***

- ***Thus, our goal is to develop non-animal alternative methods for screening and predicting the skin sensitization potential of Company materials early on in the discovery process***

REQUIREMENTS FOR THE INDUCTION OF SKIN SENSITIZATION



Critical Components of ACD

- T-cells
  - antigen specificity/immune recognition
  - activation/clonal expansion/memory response
  - pro-inflammatory cytokines
- Langerhans cells (LC)
  - major antigen presenting cell of epidermis
  - critical in induction phase of ACD
  - maturation induced changes: morphologically, phenotypically, and functionally
- Keratinocytes
  - most numerous cell in epidermis
  - role in initiation and regulation of ACD: survival of LC and regulation of LC APC ability via cytokine production

PBMC / Mo-DC

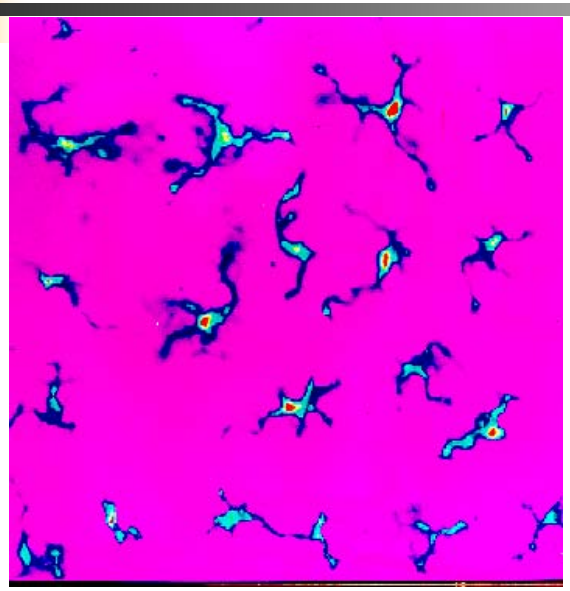
- DC generated from the adherent fraction (CD14+ monocytes) of peripheral blood mononuclear cells (PBMC)

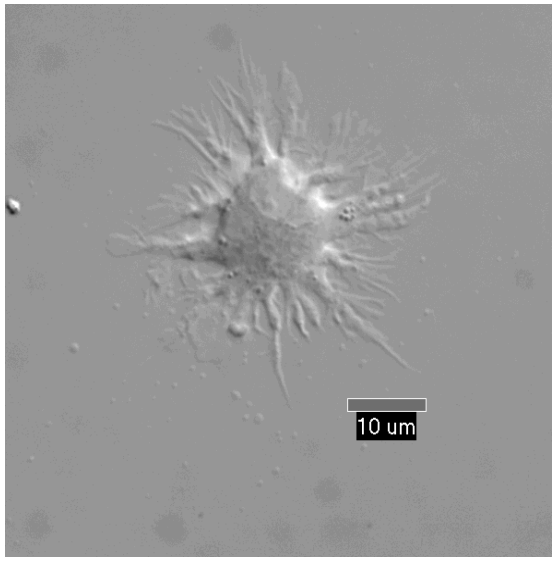
- Often referred to as PBMC-DC or Mo-DC (monocyte-derived DC)

- Peripheral blood is easy to obtain:
  - Leukopack (condensed from blood unit – yields 20-50 x 10^6 DC, cost approx $100)
  - Elutriated monocytes (commercially available, expensive, $3500 but high DC yields, 300-600 x 10^6 DC)

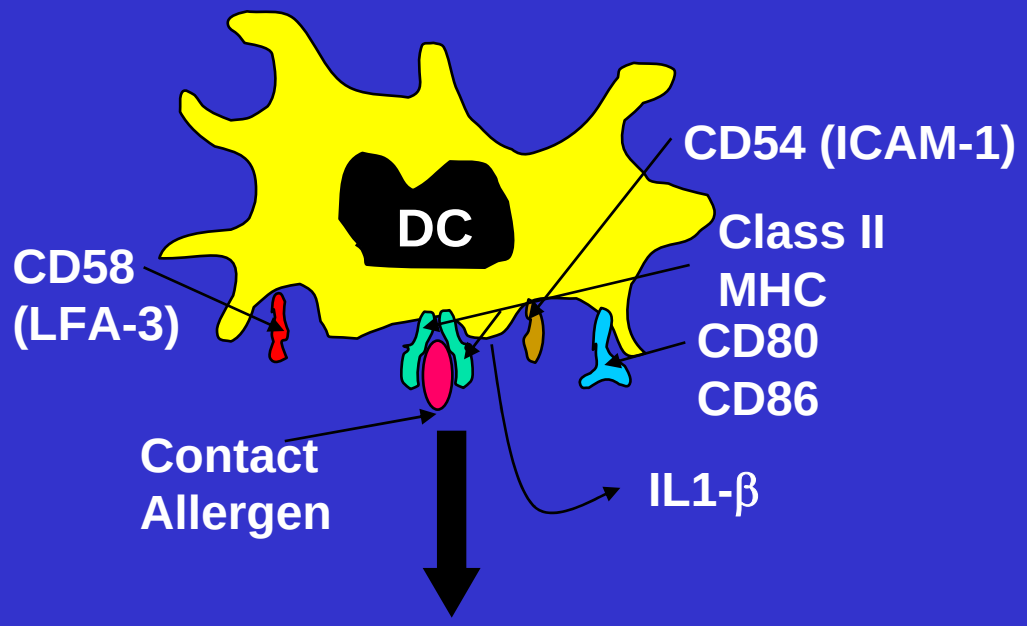
- Mo-DC share phenotypic characteristics similar to LC

Dendritic Cell Activation: A Genomics Approach

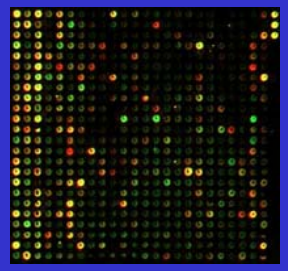




DC Activation by Contact Allergens



Identify Changes in Gene Expression



Experimental Design

Peripheral Blood Leukocytes

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Mononuclear Cells
Adherent Fraction

↓

Culture 7 Days
GM-CSF + IL-4

↓

Allergen Treatment In the Presence of Cytokines for 24 hr

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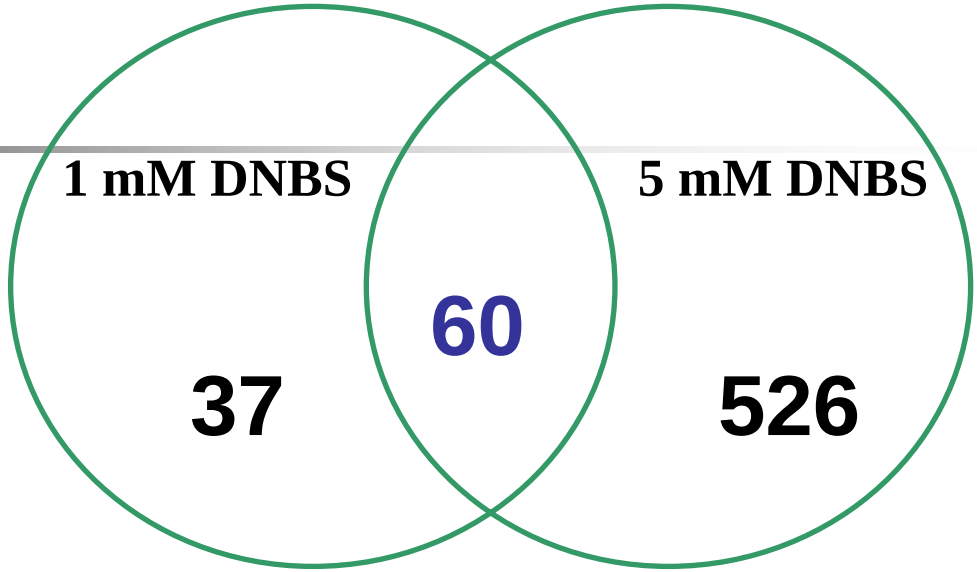
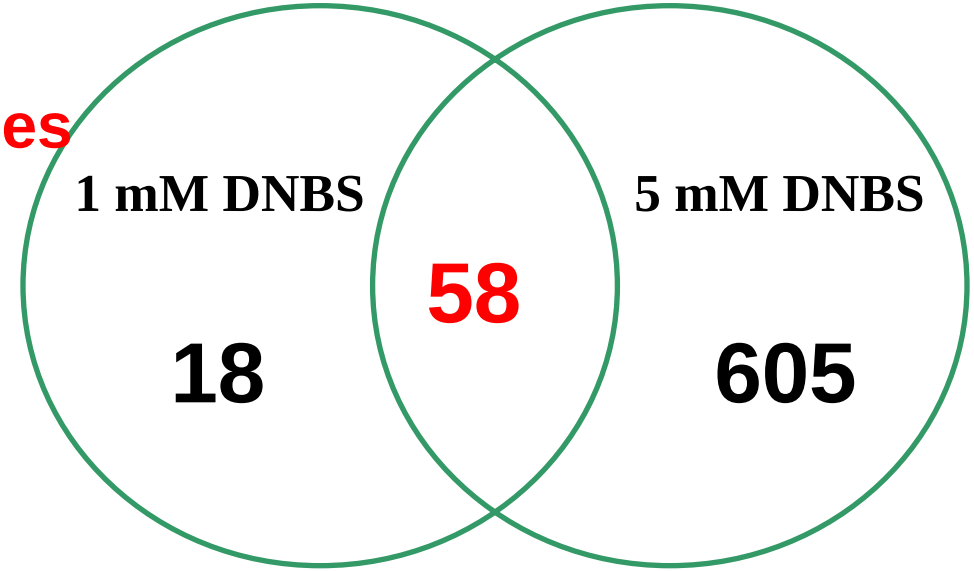
Isolate RNA for Affymetrix Analysis
Flow Cytometric Analysis
Real-time PCR Analysis

p⏱0.001

Up-Regulated Genes



Down-Regulated Genes



Target Genes of Interest

GENE	Symbol	1 mM	5 mM
Cytokine/Chemokine			
Chemokine (C-C motif) receptor-like 2	CCRL2	1.8	9.5
Chemokine (C-C motif) Ligand 2; MCP-1	CCL2	-2.8	-10.1
Chemokine (C-C motif) Ligand 23	CCL23	-1.3	-3.1
Interleukin-8	IL8	11.9	32.7
Interleukin 3 Receptor alpha subunit	IL3RX	1.6	2.2
Protein Modification/Synthesis			
Cathepsin L	CTSL	3.5	10.7
Cathepsin H	CTSH	-2	-11
Cell Surface Receptors/ Membrane Proteins			
CD86 antigen	CD86	2	2
Macrophage Lectin 2	HML2	-6.9	-18.2
Notch homolog 3	NOTCH3	13.4	27.8
Macrophage Mannose Receptor	MRC1	-1	-4.1
CD1E Antigen, e polypeptide	CD1E	-2.8	-23.5
Small Molecule Transport			
S100 Calcium biding protein A4	s100A4	-1.7	-1.4
Miscellaneous			
3-alpha hydroxysteroid dehydroenase, type III	AKR1C2	18.1	73.9
Erythrocyte membrane protein band 4.1-like 2	EPB41L2	-1.6	-5.2
Fibrinogen-like 2	FGL2	-1.1	-6.2
Interferon-stimulated gene 20kD	ISG20	2.2	10.4

Dinitrobenzenesulfonic Acid

DNBS

GENE	0.3 mM DNBS	0.6 mM DNBS	1.0 mM DNBS	2.5 mM DNBS	5.0 mM DNBS
CD86	-1.4	1	-1.4	1.5	1.6
IL-8	2.4	8.6	10.7	39.8	65.9
S100	-1.9	-2.6	-4.3	-10.9	-6.5
CCL2	-1.1	-1.4	-7.6	-8	-3.7
IL-3Rα	-1.6	1	1	1.1	3.4
HML2	-1.7	-3.9	-12.7	-36.3	-42.2
CD1e			-4.7		-51.5
ISG20	1.2	1.9	3.3	14.3	30.1
AKR1C2	2.6	9.7	15.6	42.9	229
MRC1	1.1	-1.1	-1.4	-2.3	-6.5
FGL2	-1.7	-1.7	-4.5	-5.1	-22.5
CCRL2	-1.2	1.5	1.9	2.9	5
CCL23	1.1	-3.8	-3.5	-7.4	-9.5
NOTCH 3	1.8		4.2		17.6
EPB41L2	-2.1	-4.8	-6	-6.3	-8
CTSL	1.1	1.8	1.34	6.8	9.9
CTSH	-1.9	-2.3	-4.8	-5	-5.1

29 Target Genes

- ABCA6
- AKR1C2
- ARGDIB
- BLNK
- CCL2
- CCL23
- CCL4
- CCRL2
- CD1E
- CTSH
- CYP27A1
- EPB41L2
- FCER1A
- FGL2
- G1P2
- HML2
- IER3
- IL3RAX
- MRC1
- NOTCH3
- QPCT
- RIT1
- S100A4
- SH3BP5
- SLAM
- SPN
- TRIM16
- TTRAP
- TXN

Test Chemicals

Allergens

- Eugenol – weak
- Hydroxycitronellal - weak
- Penicillin G - weak
- $NiSO_4$ - weak
- Hexylcinnamic aldehyde - moderate
- Isoeugenol – moderate
- DNBS - strong
- PLG – strong
- SADE - strong
- Hydroquinone – strong
- Diphenylcyclopropenione – extreme

Irritants

- SDS
- Benzoic Acid
- Methyl Salicylate
- Benzenesulfonic Acid

Scoring of Candidate Genes

Gene	Allergen Score	Irritant Score
ABCA6	10 / 11	1 / 4
AKR1C2	11 / 11	1 / 4
ARHGDIB	10 / 10	2 / 4
BLNK	10 / 11	1 / 4
CCL2	8 / 10	1 / 3
CCL23	11 / 11	1 / 4
CCL4	10 / 11	0 / 4
CCRL2	3 / 9	0 / 3
CD1E	11 / 11	2 / 4
CTSH	9 / 11	2 / 4

Gene	Allergen Score	Irritant Score
CYP27A1	11 / 11	1 / 4
EPB41L2	9 / 11	1 / 4
FCER1A	6 / 8	0 / 3
FGL2	10 / 11	0 / 4
G1P2	2 / 9	1 / 3
HML2	11 / 11	0 / 4
IER3	4 / 8	1 / 3
IL3RAX	5 / 8	1 / 4
MRC1	5 / 11	1 / 4
NOTCH3	11 / 11	1 / 4

Target Gene List

PRIORITY I

- Notch 3
- SLAM
- HDD2 (AKR1C3)
- Rho-diss Inhib

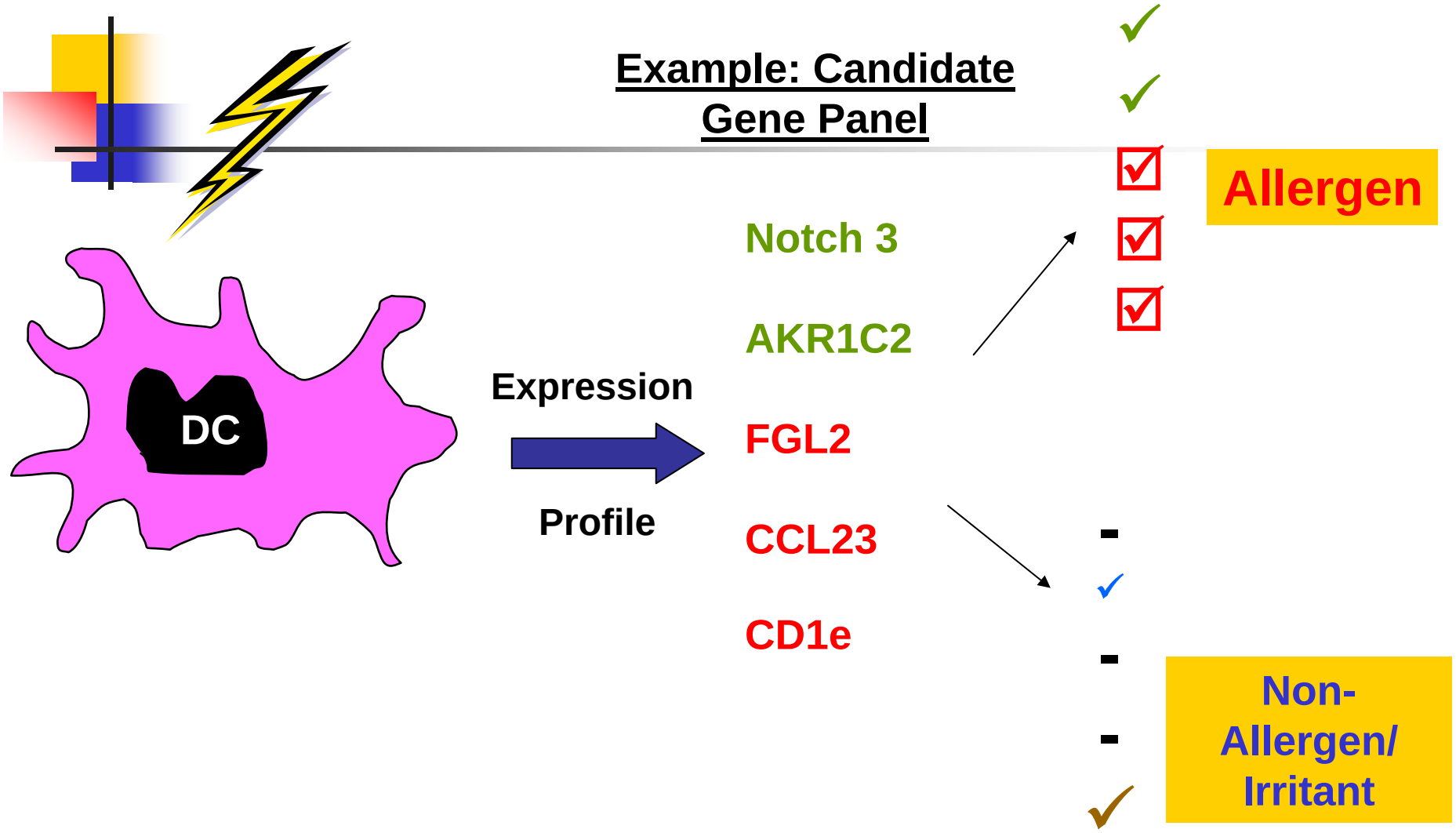
PRIORITY II

- CYP27A1
- CCL23
- CD1e
- Lectin Receptor
- ABCA6

PRIORITY III

- MRC1
- TRIM16
- CCL4
- Cathepsin H
- Protein 4.1
- BLNK
- FGL2
- CCL2
- TX1

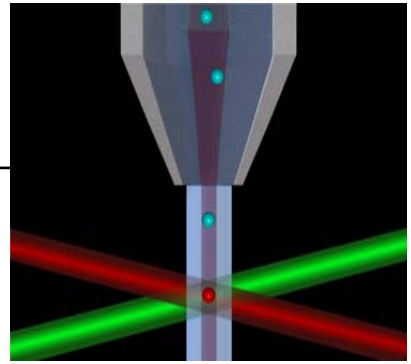
Test Chemical



Background-Summary

- Affymetrix GeneChip® Study
  - PBMC derived-DC (7d GM-CSF + IL-4)
  - 1 mM and 5 mM DNBS for 24 hr
  - Significant gene changes ($p \leq 0.001$): 60 up-regulated by both 1 & 5 mM DNBS 58 down-regulated by both 1 & 5 mM DNBS
  - Toxicology Letters (2004), 150:301-316
- Verification by real time PCR and examination of donor-to-donor variability and dynamic range
  - 60 genes selected from 118 tested in 2 DNBS dose response studies
- Examination of allergen specificity
  - 50 of those 60 examined in a single donor with 3 allergens, 1 irritant and 1 non-allergen
- 29 genes selected for further examination
  - Currently using real time PCR
  - Need for a high-throughput screening method

Luminex xMAP Technology

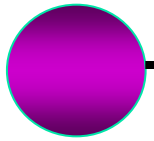


Based on the principles of flow cytometry

- **Polystyrene microspheres internally dyed with spectrally distinct fluorchromes define a set of signature beads (bead mix)**
- **Each set of signature beads carries a specific detection reagent (e.g. oligonucleotide probe) on its surface**
- **Analytes contained in added samples bind to detection reagents on beads**
- **A reporter coupled with another fluorochrome quantifies the reaction on the bead surface**
- **The biomolecular interactions are quantified by the Luminex analyzer while passing the laser beams**

Luminex xMAP Technology

Radix custom xMAP Microspheres with oligonucleotide probe



CCCGGGACCAATCACCATGGACCTT

Oligonucleotide Probe Selection

- **Strategy: Select the probe that best correlates with the Affymetrix signal**
- **Algorithm for probe selection developed by P&G Biostatistician (copyrighted)**
  - **examines the 'Perfect Match' oligos from Affymetrix Genechip® probe pairs**
  - **correlation of individual probes to Affy signal value**
  - **correlation of individual probes to other probes within the same probe set**

Methods

CHEMICAL TREATMENT of PBMC- DC for 24h

↓

RNA ISOLATION: Isolate total RNA with TRIZOL, clean-up with RNEasy kit, quantitate by spectrophometer

↓

cRNA TARGET GENERATION : Reverse transcribe RNA into cDNA. Generate biotin-labeled cRNA with Enzo Bioarray RNA Transcription kit then fragment.

↓

HYBRIDIZE TARGET TO BEADS and STAIN with SA-PE (Amplified Staining with biotinylated anti-SA antibody)

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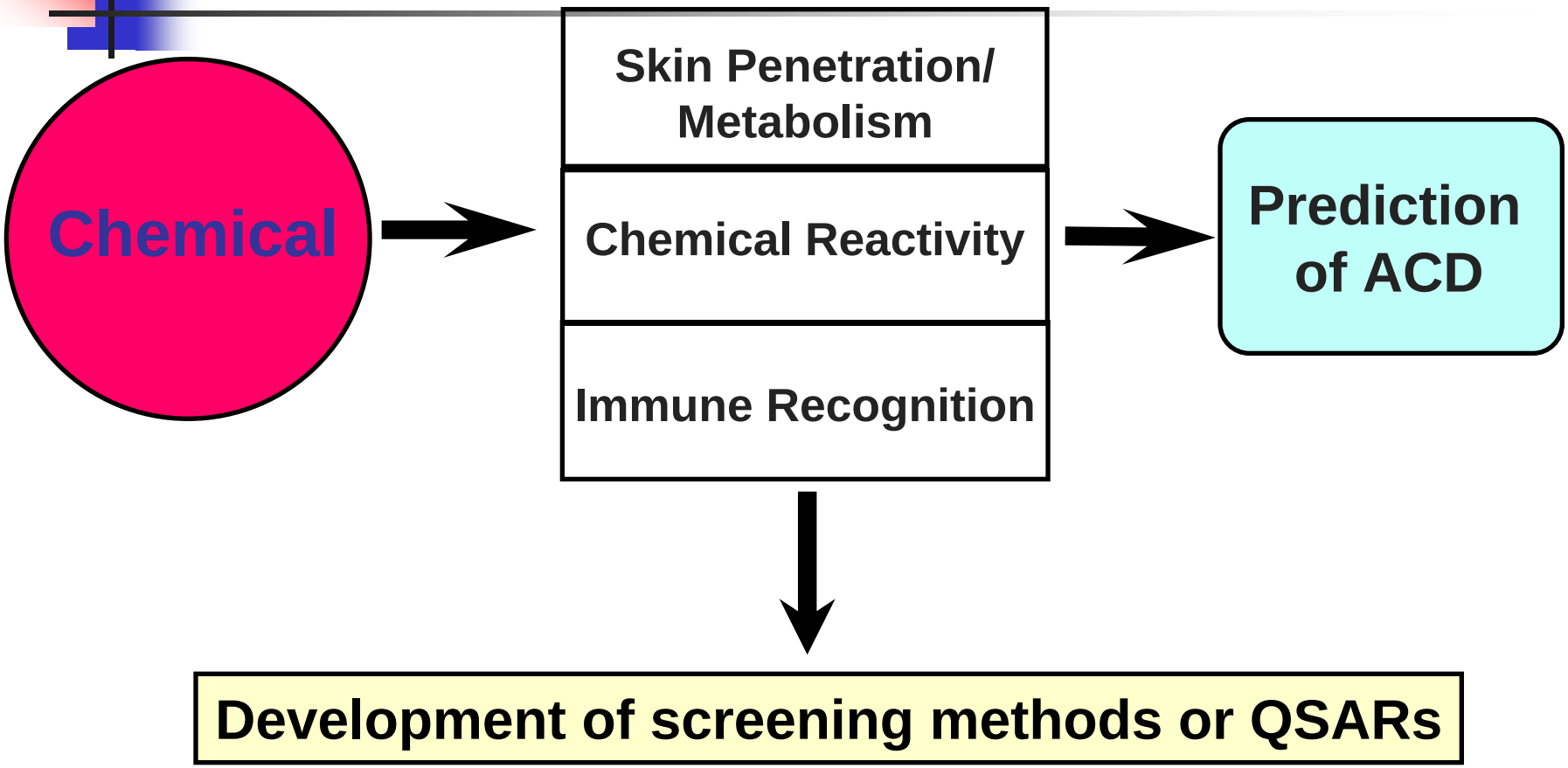
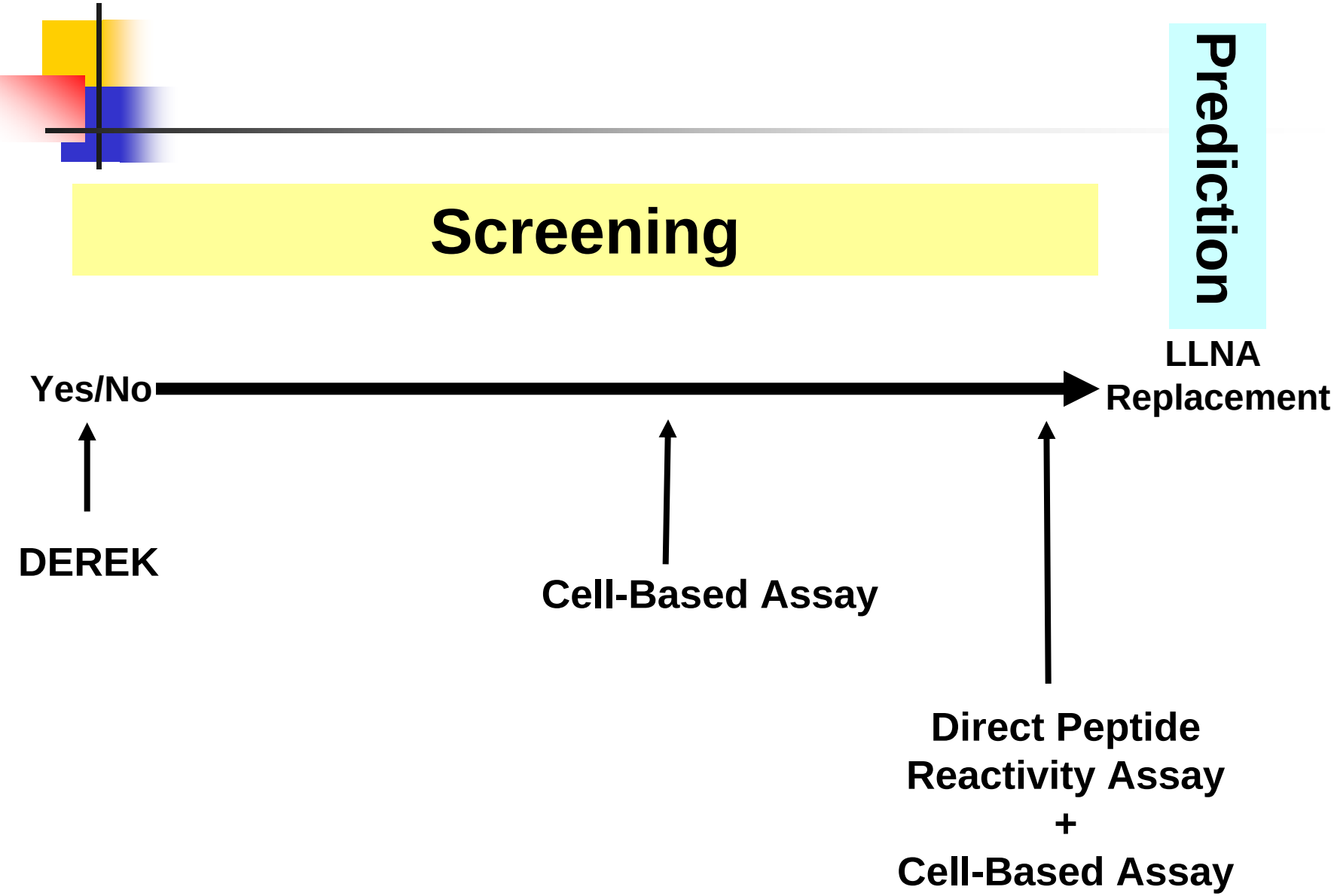
DATA ANALYSIS: Read beads on Bio-Rad Bio-Plex (Luminex Analyzer)

Mechanistic Based *In Vitro* Approach for Skin Sensitization



Development of Test Battery for Skin Sensitization Testing



Next Steps

- Analyze target genes with a test set of chemicals
- Further prioritize gene list
- Investigate new technology platform – Luminex/Radix
- Incorporate Cellomics technology to increase throughput (cytotoxicity)

Acknowledgements

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