XCellR8
Redefining testing

# Your Guide to GARD

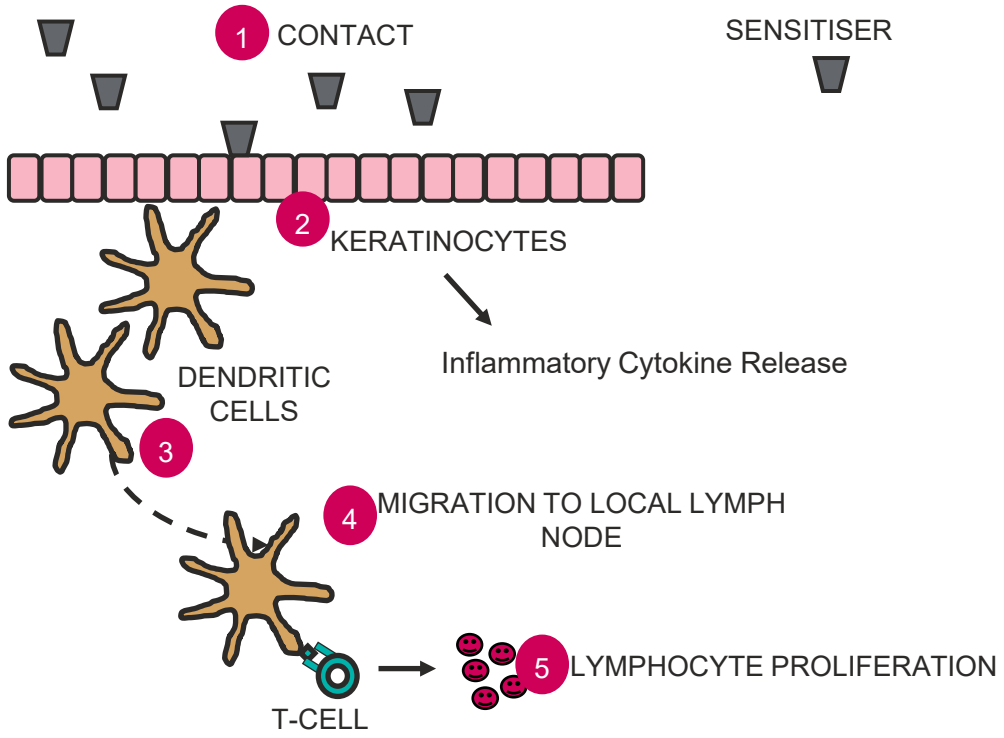
## NEW GENERATION SKIN SENSITISATION TESTING TECHNOLOGIES EXPLAINED

Dr Carol Treasure

15th September 2020, Webinar

# Hazard identification and potency assessment for human skin sensitisation: the role of GARD®skin and GARDpotency

# Skin sensitisation leading to allergy - adverse outcome pathway (AOP)




## KEY EVENTS IN SKIN SENSITISATION AND RELATED TESTS

1. Contact
   (Direct Peptide Reactivity Assay – **DPRA**)
2. Release of Pro-Inflammatory Cytokines by Keratinocytes (**KeratinoSens™**)
3. Dendritic Cell Activation/Maturation
   (human Cell Line Activation Test – **h-CLAT, GARD®skin**)
4. Migration
5. T-cell Proliferation
   (Local Lymph Node Assay - LLNA)

A truly global effort! – tests developed in Switzerland, Sweden, US and Japan

Current regulatory guidance favours "2 out of 3" approach

# *In vitro* skin sensitisation testing

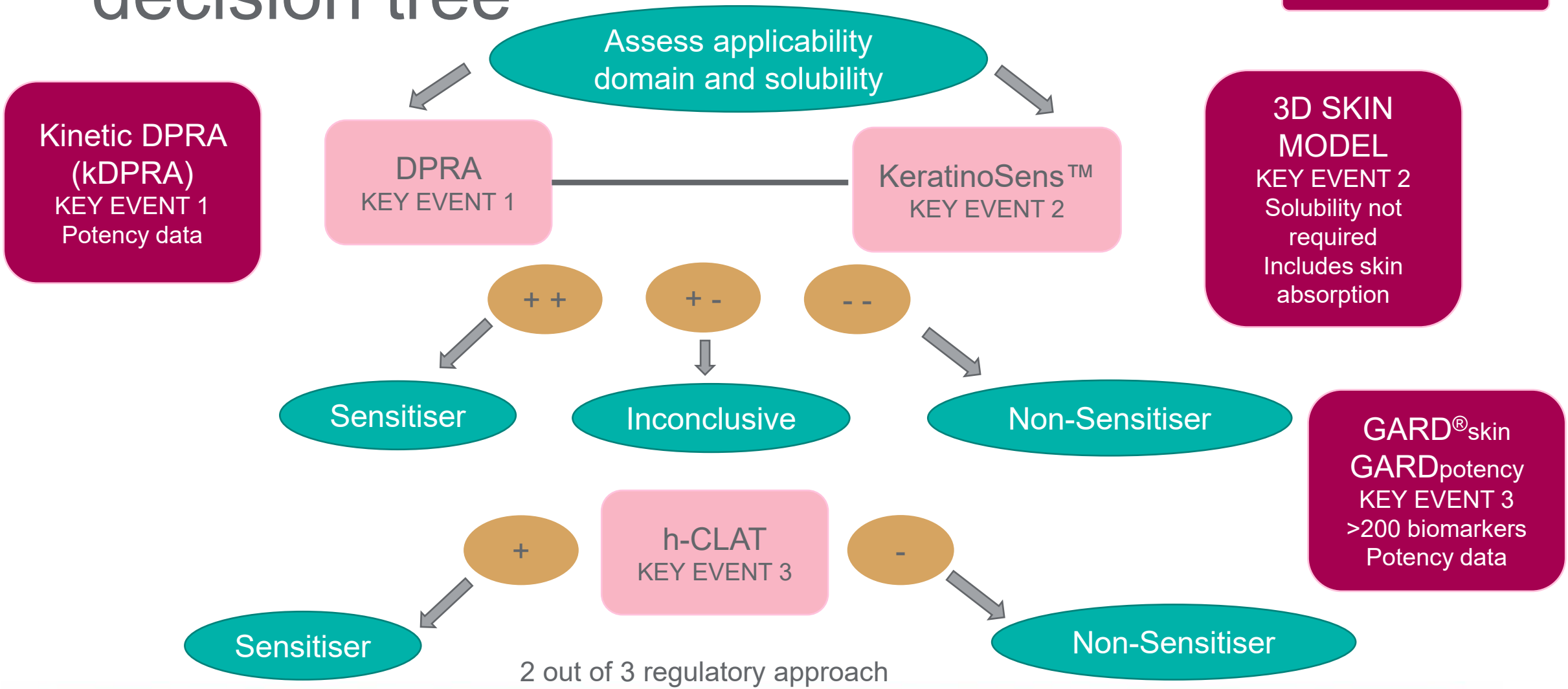
## WHY 2 OUT OF 3?

*In vitro* tests replacing a complex *in vivo* pathway;
Advanced mechanistic data on human response.

- DPRA (OECD TG 442c)
- KeratinoSens™ (OECD TG 442d)
- h-CLAT (OECD TG 442e)

# *In vitro* skin sensitisation testing – decision tree

KEY:
REGULATORY
NON-REGULATORY



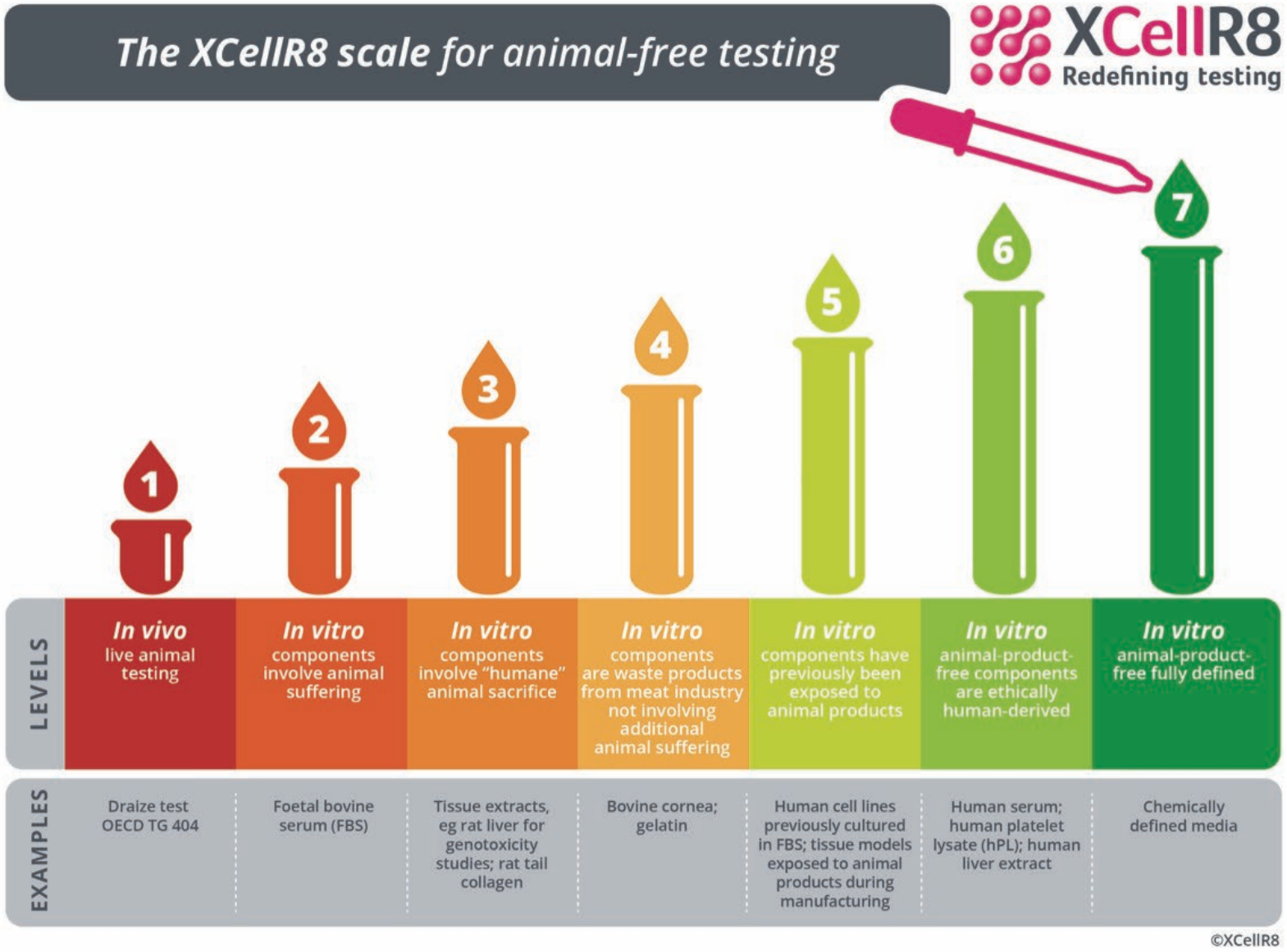
# Why are potency predictions important?

- Recent article in Chemical Watch: over 50% REACH substances may require animal tests for skin sensitisation
  - Incompatibility with *in vitro* tests (eg UVCBs)
  - Potency data required to distinguish between GHS category 1A and 1B sensitisers
- Current *in vitro* regulatory tests provide limited potency data
- 2 ways to address this:
  - Drop sub-categories 1A and 1B, or:
  - Include potency tests in OECD Guideline on Define Approaches (DA) for Skin Sensitisation, once a potency TG is available.
- 47,000 skin sensitisation tests carried out on animals in EU in 2017, for research and regulatory testing – guinea pig tests as well as Local Lymph Node Assay (LLNA)

# How the new animal-product-free version of GARD®skin can help support vegan tested claims

# What is truly animal-free testing?

- All *in vitro* tests are not equal in terms of “animal-free” status
- Most *in vitro* methods use animal components
  - Fetal bovine serum
  - Tissue extracts
  - Antibodies
- Reasons are largely historical
- Truly animal-free testing needs to be animal-*product*-free
- Driven by:
  - **Science:** greater human relevance and higher reproducibility.
  - **Ethics:** consumer and industry demand for sustainable, ethical products (*and* ethical testing), eg vegan products require vegan-compliant testing




Adaptations of skin sensitisation tests have achieved Levels 5-7 (green zone)

# GARD®skin APF

- Preliminary validation data will be shared by Senzagen
- Complete: replacement of FBS with human serum
- Ongoing: animal-free antibodies

**Applications:**

- Product development (non-regulatory screening)
- Vegan tested claims
- Companies adopting APF conditions for scientific and ethical advantages
- Exclusively available through XCellR8

# Thank you!

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# Your guide to the GARD platform

*In vitro* sensitization testing using Genomics and Machine Learning

Andy Forreryd, PhD

September 15, 2020

SENZA GEN

# GARD™ assay portfolio

## For skin and respiratory sensitization testing

GLP

### Skin Sensitization

#### GARD™skin
200 genes

A robust *in vitro* assay to identify potential chemical skin sensitizers with over 90% prediction accuracy

#### GARD™ potency
51 genes

An add-on *in vitro* test to GARDskin for potency classification according to GHS/CLP (1A or 1B)

### Respiratory Sensitization

#### GARD™ air
28 genes

The first *in vitro* assay capable of identifying chemical respiratory sensitizers

#### GARD™ skin Medical Device
200 genes

A robust and accurate *in vitro* assay to test for skin sensitizers in Medical Device extracts according to ISO 10993-12: 2012

New

#### GARD™ skin Dose-Response
200 genes

Quantitative potency assessments with high correlation to LLNA EC3 values and human potency

More information available at
**Senzagen.com/webinars**

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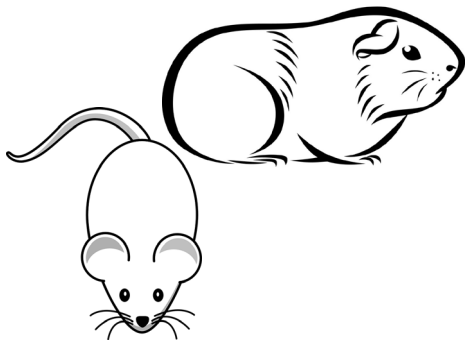
# Ensuring reliable results and safe products

## Replacing animal testing with modern technology

Human cells
Genomics
Machine learning

### Traditional testing:
*In vivo*

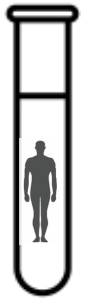


70-75%

Accuracy against human data

- Long turnaround time
- Expensive
- Ethical considerations

### First generation
*In vitro*



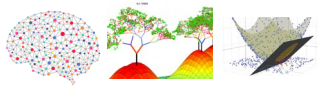
75-80%

Accuracy

- Single biomarkers
- Limited mechanistic information
- No potency information
- Used within defined approaches

### GARD: Next generation
*In vitro*


$$maximize\ f(c_1 \dots c_n) = \sum_{i=1}^{n} c_i - \frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n} y_i c_i \left(\varphi(x_i) * \varphi(x_j)\right) y_j c_j$$
$$= \sum_{i=1}^{n} c_i - \frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n} y_i c_i k(x_i, x_j) y_j c_j$$
$$Subject\ to \sum_{i=1}^{n} c_i y_i = 0, and\ 0 \le c_i \le \frac{1}{2n\lambda}\ for\ all\ i$$




90-95%

Accuracy

- Biomarker signatures & Toxicity pathways
- Potency information
- Reliable & mechanistically relevant results

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# The GARD™ technology platform

## Human relevant cells in combination with Genomics and Machine Learning

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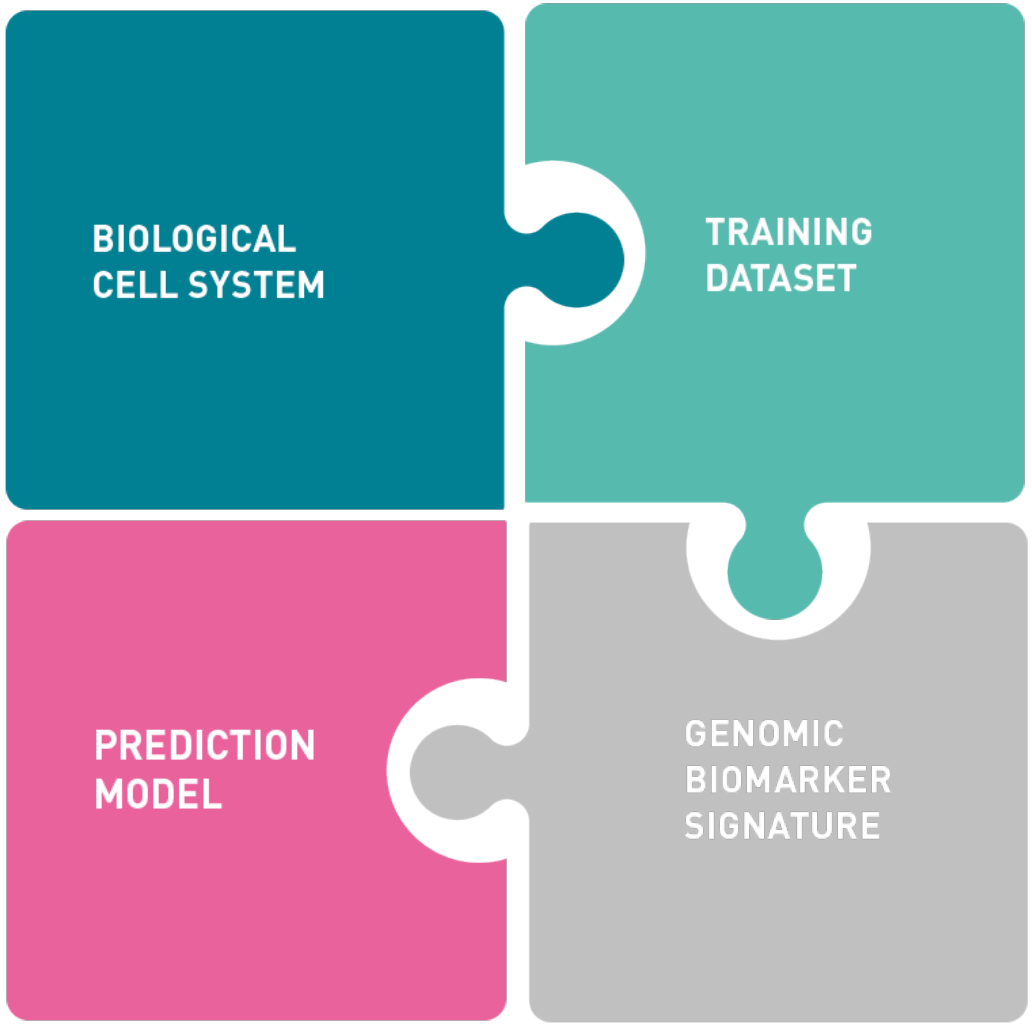
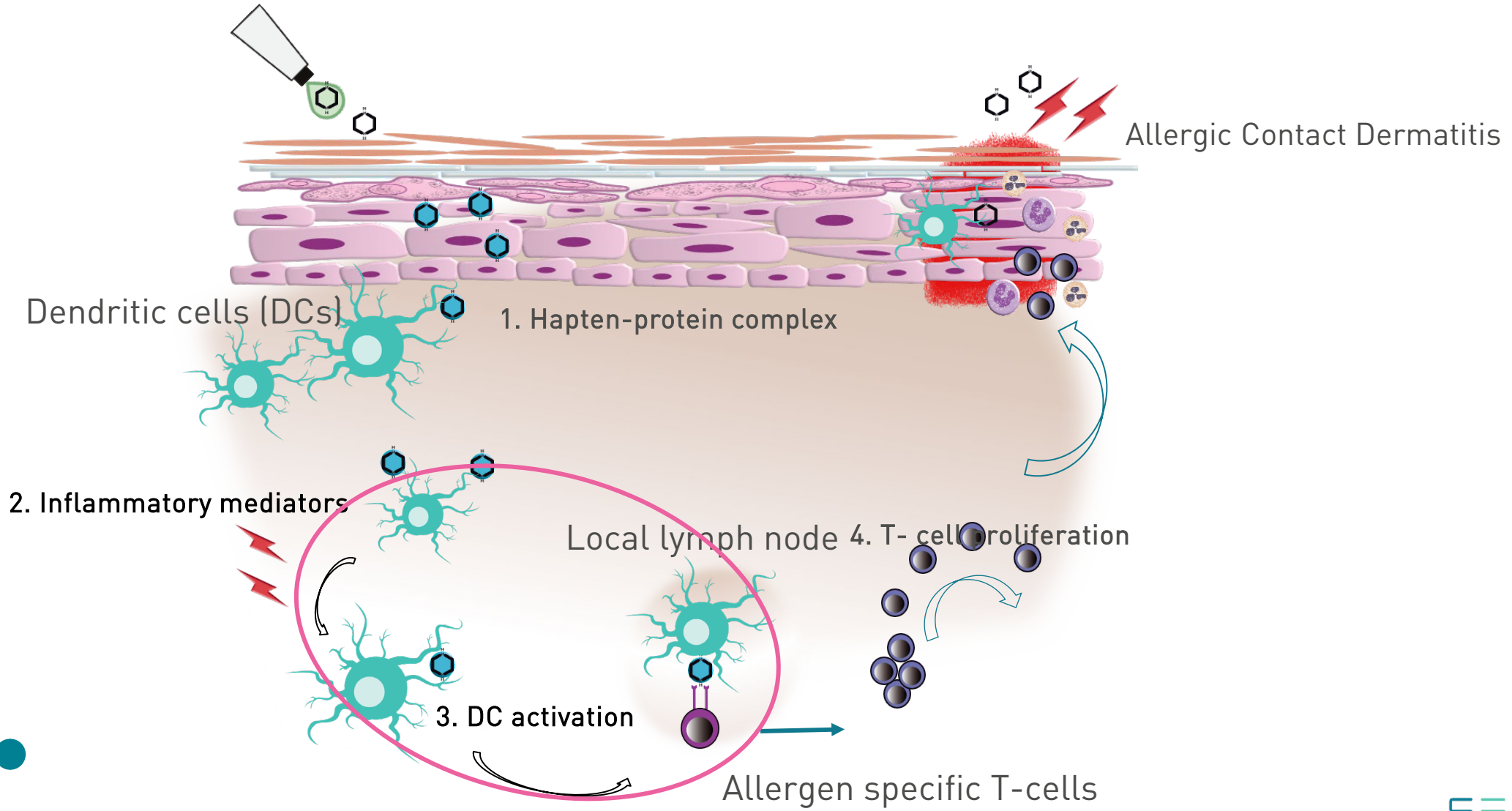
# The GARD™ technology platform – how it works



# The GARD™ technology platform – how it works

## Based on a dendritic-like cell line - SenzaCells™




BIOLOGICAL
CELL SYSTEM

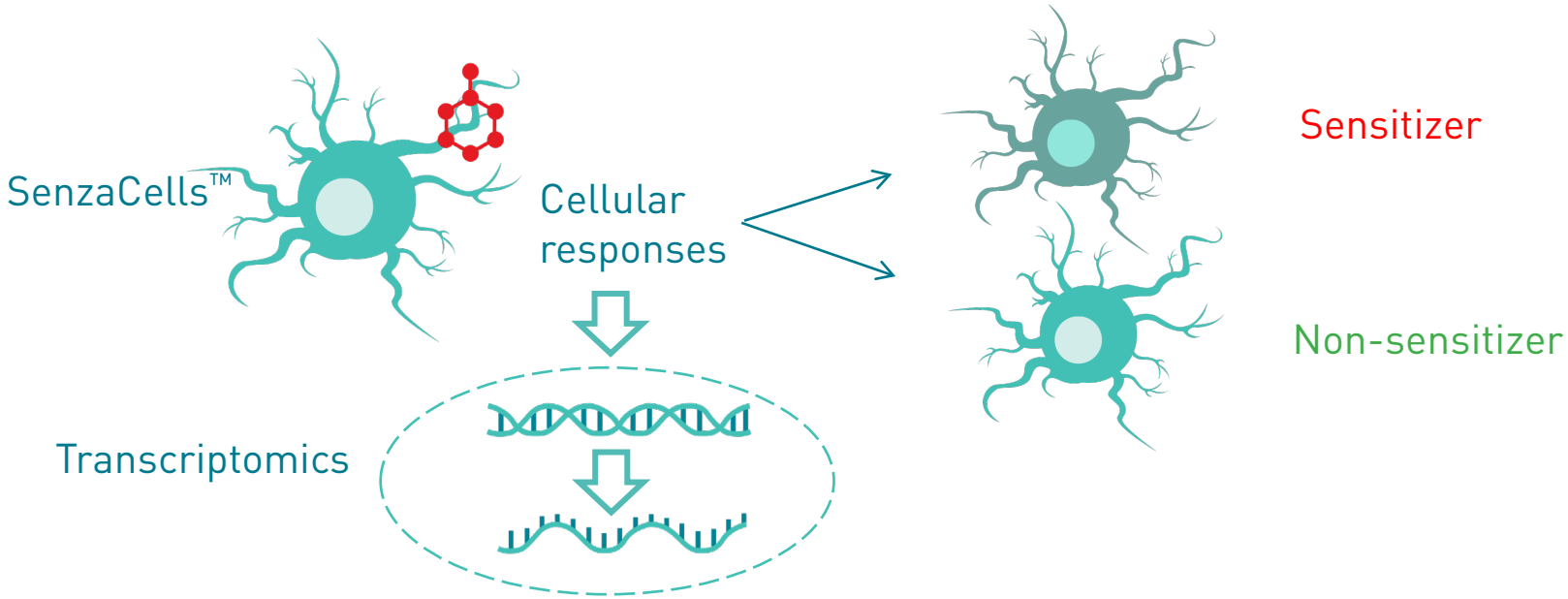
SENZA
GEN

# The GARD™ technology platform – how it works

## Transcriptomic read-out of the biological response

### Assay Development: Hypothesis

- There should exist genes and pathways in DCs that are differentially expressed depending on the stimuli.
- Such genes could be used as predictive tools.




BIOLOGICAL CELL SYSTEM

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# The GARD™ technology platform – how it works

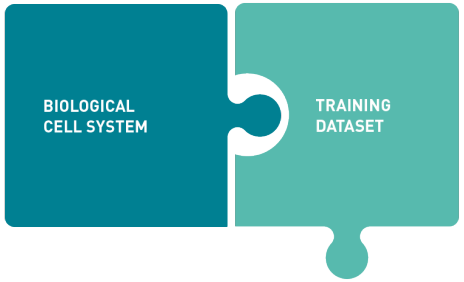
## Reference compounds are used to create whole-genome training datasets

A human dendritic-like cell line **(SenzaCells)** were stimulated with a Reference set of chemicals.

Transcriptional levels of the genetic material was assessed with microarray technology

**=29.000 genes/sample**

| *Sensitizers* | *Non-sensitizers* |
|---|---|
| 2,4-Dinitrochlorobenzene | 1-Butanol |
| Oxazolone | 4-Aminobenzoic acid |
| Potassium dichromate | Benzaldehyde |
| Kathon CG (MC/MCI) | Chlorobenzene |
| Formaldehyde | Diethyl phthalate |
| 2-Aminophenol | Dimethyl formamide |
| 2-nitro-1,4-Phenylendiamine | Ethyl vanillin |
| p-Phenylendiamine | Glycerol |
| Hexylcinnamic aldehyde | Isopropanol |
| 2-Hydroxyethyl acrylate | Lactic acid |
| 2-Mercaptobenzothiazole | Methyl salicylate |
| Glyoxal | Octanoic acid |
| Cinnamaldehyde | Propylene glycol |
| Isoeugenol | Phenol |
| Ethylendiamine | p-Hydroxybenzoic acid |
| Resorcinol | Potassium permanganate |
| Cinnamic alcohol | Salicylic acid |
| Eugenol | Sodium dodecyl sulphate |
| Penicillin G | |
| Geraniol | |

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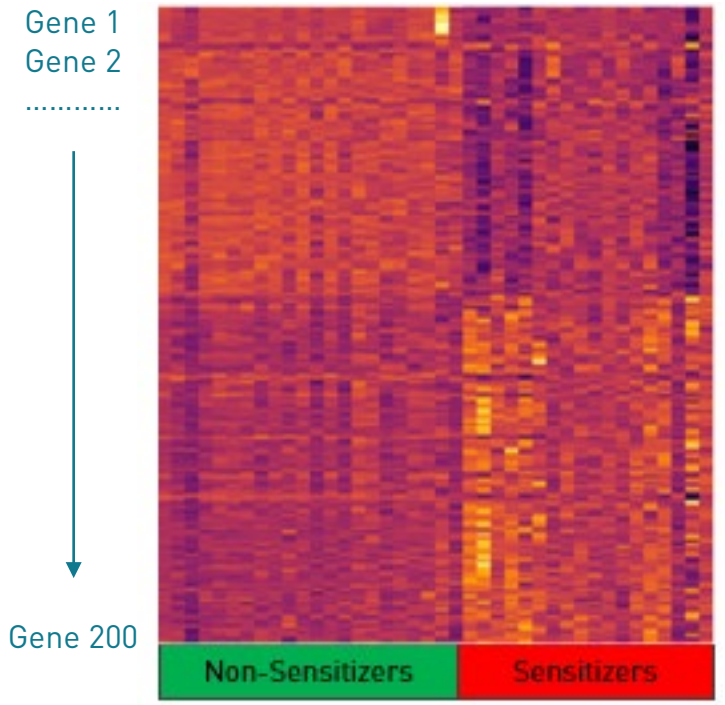
# The GARD™ technology platform – how it works

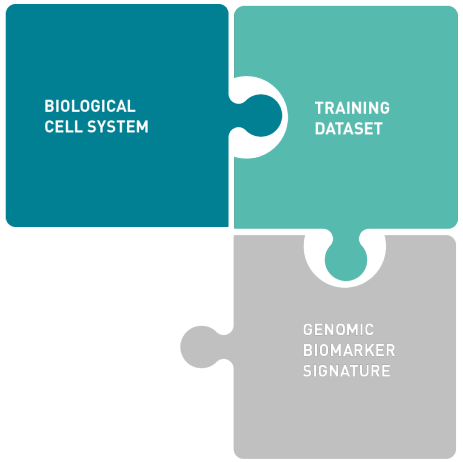
## Data-driven biomarker signature identification

### Data-driven biomarker identification

Data analysis identified differentially regulated genes in cells stimulated with **Skin sensitizers** or **Non-sensitizers.**

### GARD prediction signature





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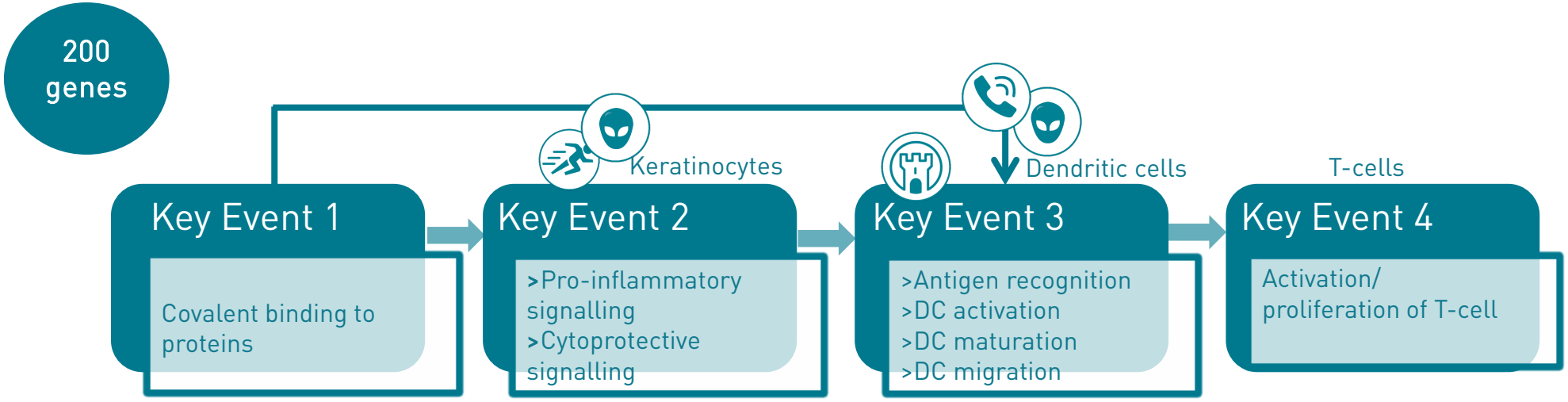
# The GARD™ technology platform – how it works

## Biomarker signature cover mechanistically relevant pathways




Captures events downstream of **KE1**

**Metabolic activity & identifies pre/pro haptens**
*ALDH*
*NAT-1*
*CYP - Cytochrome p-450*

**> Keap1-Nrf2-ARE pathway & AHR signalling**
*NQO1*
*HMOX1*
*Thioredoxin reductase I*

**> Pro-Inflammatory cytokines mediating e.g. TNFα, INFγ, IL-8**
*FAS*
*MAP2KI*
*COX20*
**> Inflammasome**
*NLRP*
*PSTPIP1*

**> DC migration & maturation**
*CD86*
*MAPK- activation*
*PKA- and GPCR- mediated signalling*

**> Antigen recognition & Innate immune activation**
*TLR-4*
*TLR-6*
*RXRA – retinoic X receptor*
*NLRP*
*PSTPIP1*
**> Self-defence mechanisms**
*C3a/C5a-activation pathways*

**Covers the 3 Key steps for T-cell activation:**
Antigen presentation
Co-stimulation
Cytokine secretion

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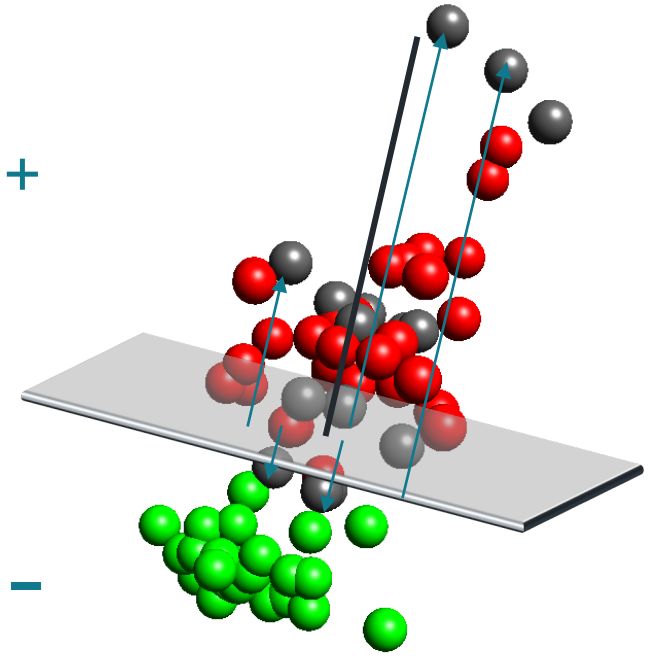
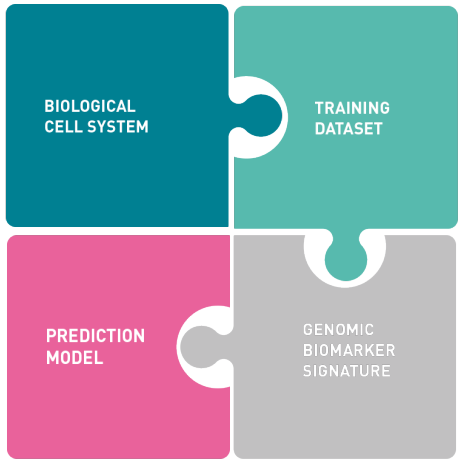
# The GARD™ technology platform – how it works

## State-of-the-art machine learning provides transparent classifications

Sensitizer (Train)
Non-Sensitizer (Train)
Unknown




Decision value > 0 = Sensitizer
Decision value < 0 = Non sensitizer




**Fixed Formula:**
Gene 1 x weight 1
Gene 2 x weight 2
Gene 3 x weight 3
....
Gene n x weight n

All genes contribute to the final classification

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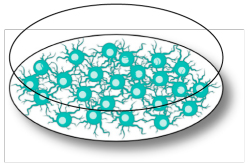
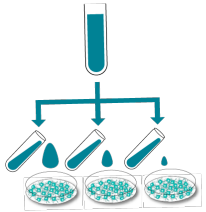
# How to GARD™ your products in 6 Steps

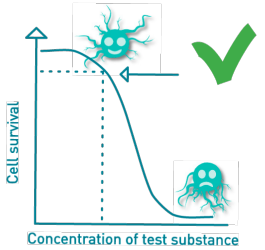
## 1 GARD Input Finder



Grown SenzaCells

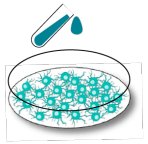


Add different concentrations of the test substance to the cells

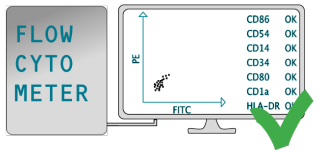



Determine the concentration of the test substance where the cells react and 90% survive

## 2 GARD Main Stimulation

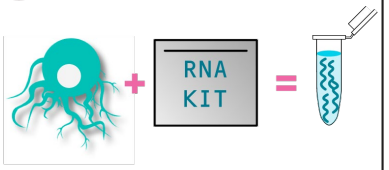


Take test substance at determined concentration and add to fresh batch of cells



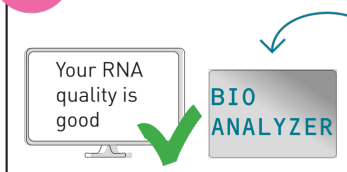

Quality control of the cells

## 3 RNA extraction

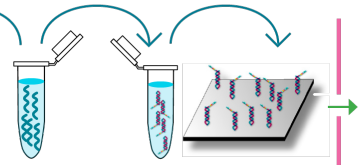



Extract RNA from the cells

## 4 Gene expression profiling



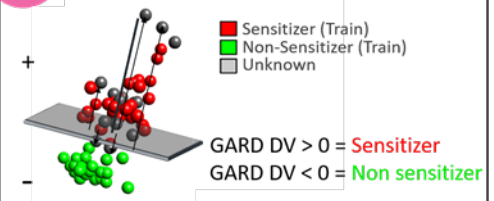

Check the RNA quality



Mix the isolated RNA with reporter probes and load onto a cassette

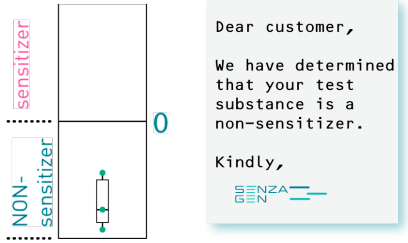

Analyze the probe cassette to quantify the RNA

## 5 GARD data analysis application




Upload the results to the GDAA web app. One press of the button and the algorithm crunches the data

## 6 Results




The results are yours!

# GARD™ assay portfolio

## For skin and respiratory sensitization testing

### Skin Sensitization

#### GARD™skin
200 genes

A robust *in vitro* assay to identify potential chemical skin sensitizers with over 90% prediction accuracy

#### GARD™ potency
51 genes

An add-on *in vitro* test to GARDskin for potency classification according to GHS/CLP (1A or 1B)

#### GARD™ skin Medical Device
200 genes

A robust and accurate *in vitro* assay to test for skin sensitizers in Medical Device extracts according to ISO 10993-12: 2012

#### GARD™ skin Dose Response
200 genes

Quantitative potency assessments with high correlation to LLNA EC3 values and human potency.

### Respiratory Sensitization

#### GARD™ air
28 genes

The first *in vitro* assay capable of identifying chemical respiratory sensitizers

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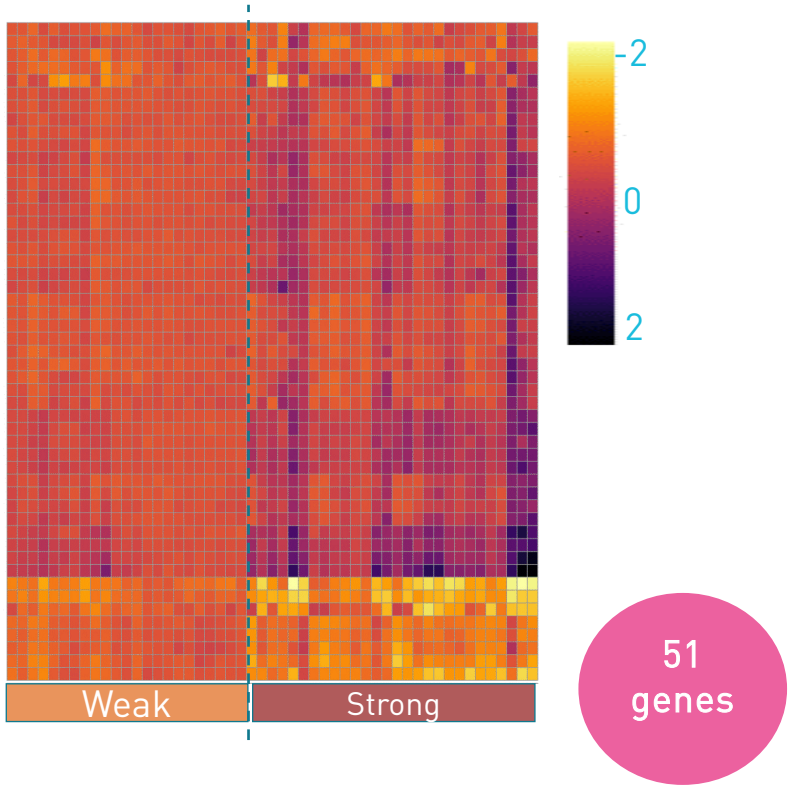
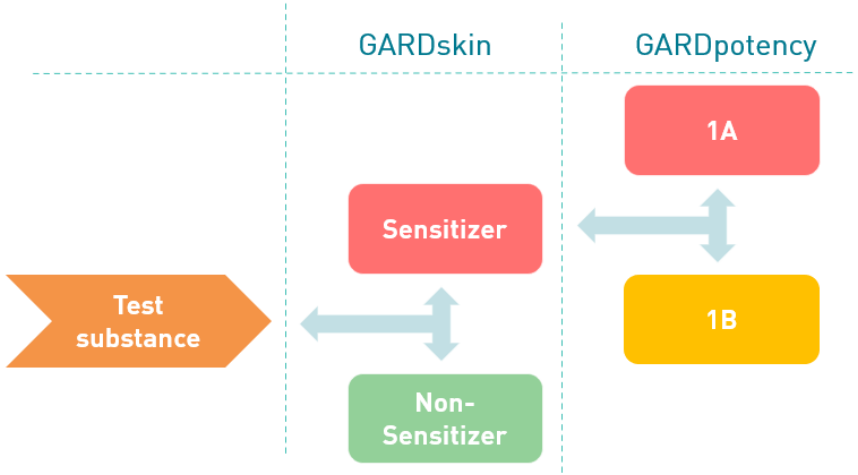
# The GARD™ potency assay

## Sub-categorization of sensitizers according to GHS/CLP (weak/strong)

### GARDpotency prediction signature



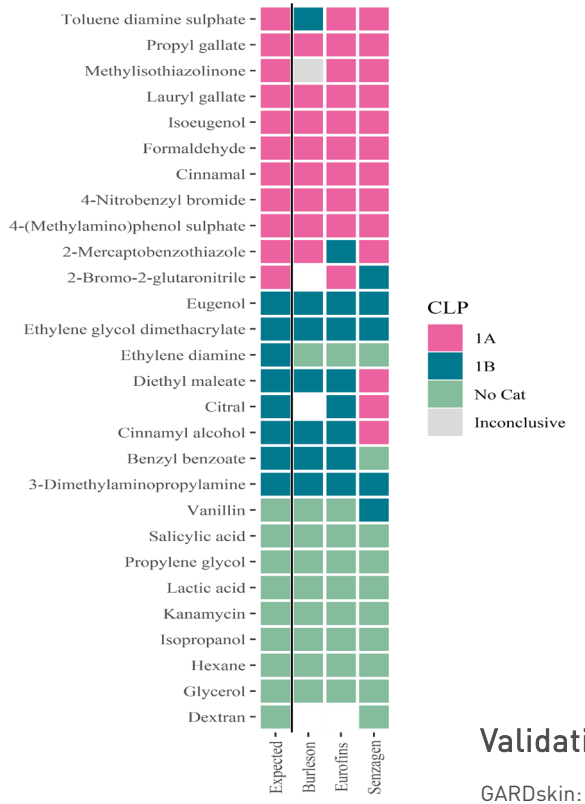

- GARDpotency classifies skin sensitizers according to GHS/CLP (strong/weak) using a complementary biomarker signature of 51 genes.
- Recommended to be used within a tiered approach:



# Regulatory compliance

## OECD validation & REACH registrations

- GARD™skin/potency are included in the OECD TGP (4.106). Under EURL ECVAM review and can already now be used as **WoE in REACH dossiers.**




### Results from validation study submitted to EURL ECVAM:

GARDskin accuracy: **94%**

GARDpotency accuracy: **89%**

The tiered testing strategy accuracy: **86%**

### Validation studies published in peer-reviewed scientific journals:

GARDskin: Published in Johansson et al. (2019), Validation of the GARD™skin assay for assessment of chemical skin sensitizers - ring trial results of predictive performance and reproducibility. ***Toxicological Sciences.***

GARDpotency: Published in Gradin et al. (2020), The GARD™potency Assay for Potency-Associated Subclassification of Chemical Skin Sensitizers - Rationale, Method Development and Ring Trial Results of Predictive Performance and Reproducibility. ***Toxicological Sciences.***

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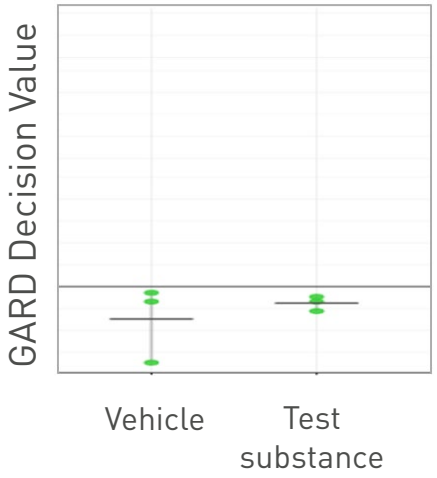
# GARD - Applicability for challenging samples

## A range of solvents enable for testing also of samples with low water solubility

### Background

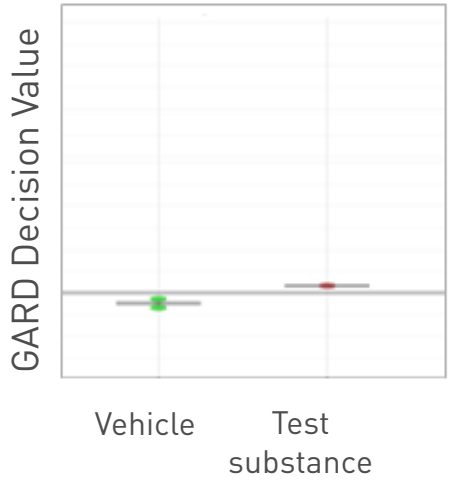
- Select solvent and concentration to increase bioavailability and avoid false negative classifications.
- **Available solvents:** Acetone | DMF | DMSO | Isopropanol | Ethanol | Glycerol | Olive oil | Sesame oil
- **GARD is highly sensitive:** An input concentration < 100 uM required to detect all sensitizers.

### Vehicle: DMSO



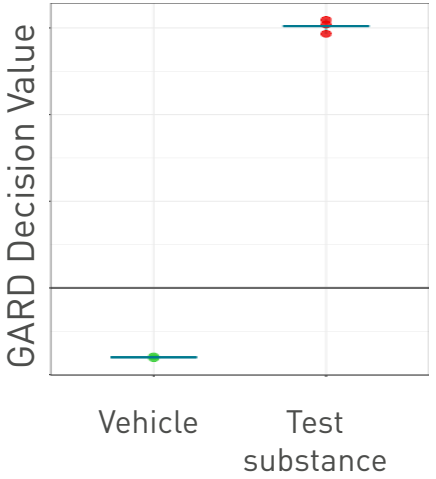

Not soluble

### Vehicle: Water




Not soluble

### Vehicle: DMF/Glycerol




Soluble

### Example

- Testing of 7 hydrophobic UVCB samples (Unknown or Variable composition). No false negative, 6/7 consistent with available animal data.

# GARD™skin APF

Animal Product-Free

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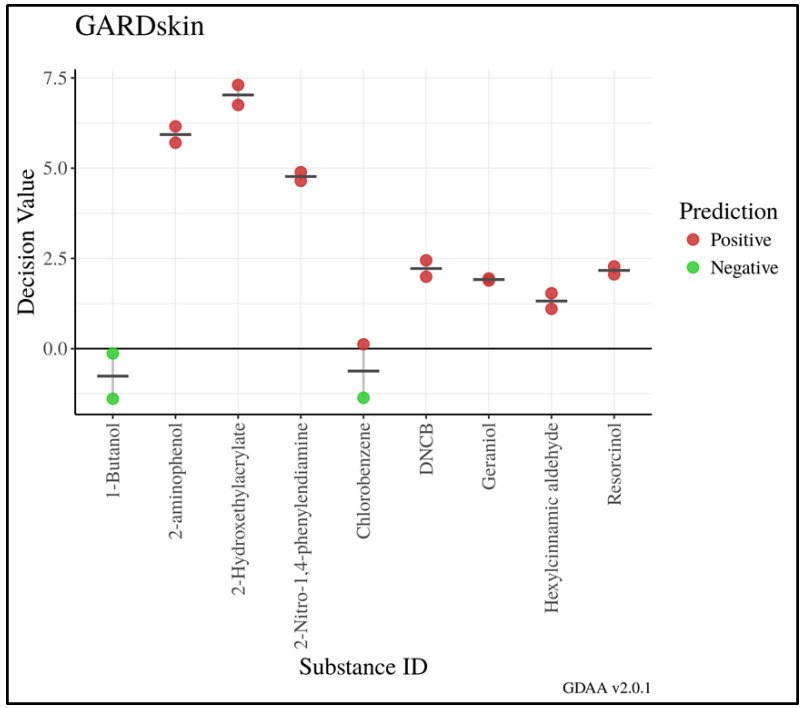
XCellR8
Redefining testing

# GARD™skin - adaption to Animal Product Free (APF)

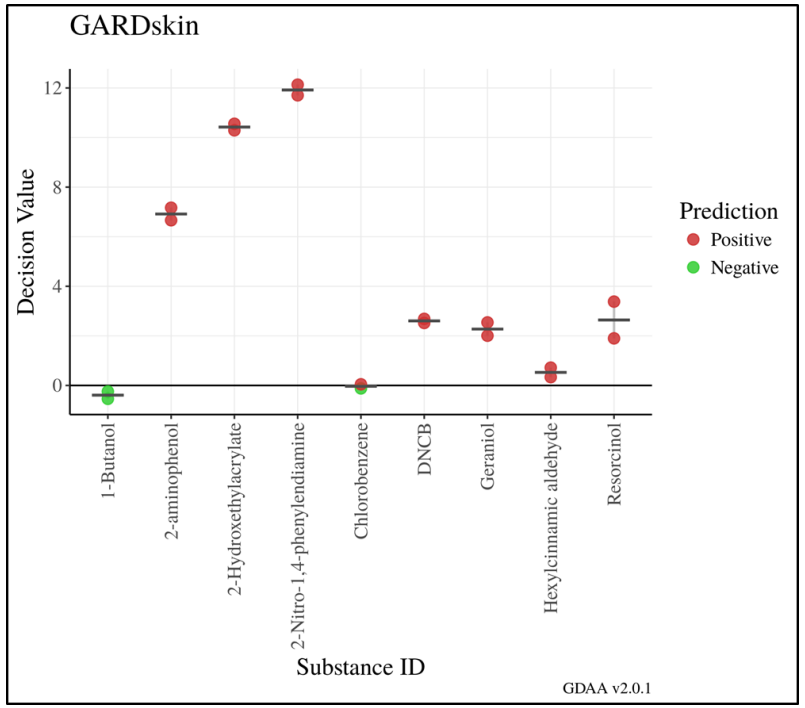
## Background

- Replace animal-derived test components with non-animal products.
- **Step 1**: Replace FBS with human serum. Perform testing in GARDskin with a proficiency set of compounds (n=9).
- **Step 2:** Evaluate animal-free antibodies for cell maintenance QC. **Ongoing work!**

SenzaCells cultivated in FCS




SenzaCells cultivated in human serum



# GARD™ summary

## Unique skin sensitization test combination

### Complex mixtures, fragrances, surfactants...

GARDskin for skin sensitizing hazard prediction with human relevance and high accuracy:

- Expertise with a broad range of difficult-to-test samples.
- For R&D or as WoE in regulatory testing.
- Use it alone or combine with other GARD tests for potency assessment.

**Difficult-to-test samples**

### Quantitative potency assessment

Use GARDpotency or GARDskin Dose-Response depending on your needs:

- GHS/CLP classification 1A or 1B.
- Potency ranking of candidate ingredients.
- Potency measurement that can be extrapolated to LLNA EC3 values and human potency category 1-6.

**Potency assessment**

GARDskin and GARDpotency are included in OECD TGP 4.106 and currently under scientific peer review.
GARDskin and potency results can be already used as WoE in REACH dossiers.

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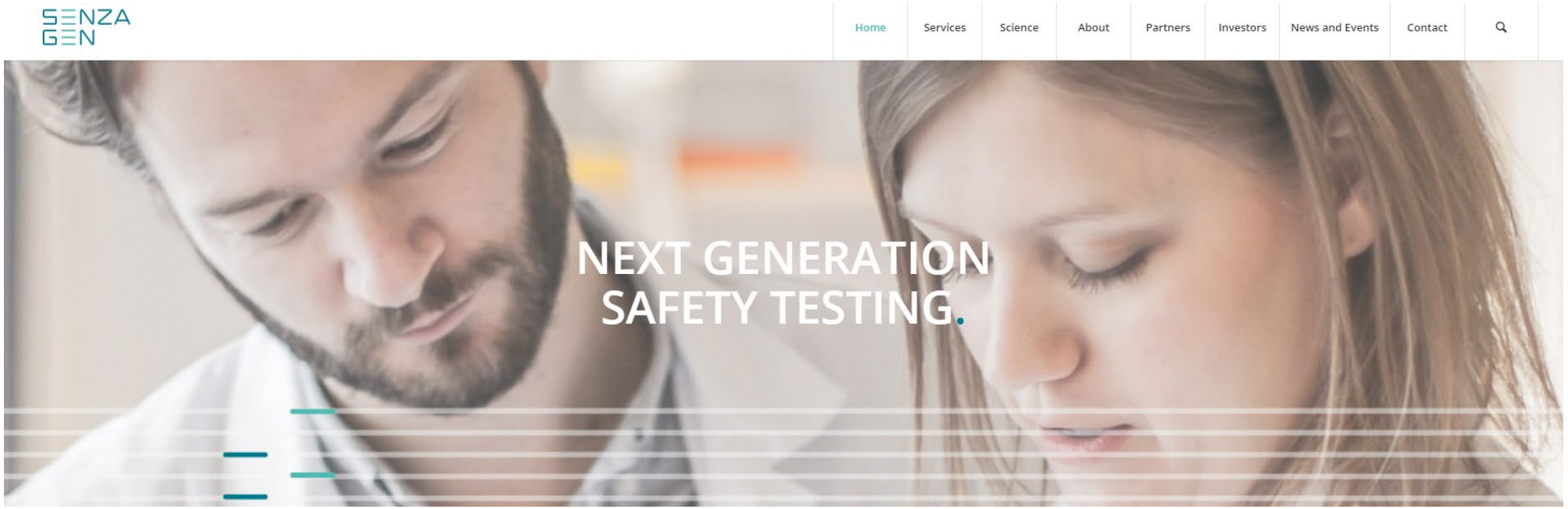
# Contact us

www.senzagen.com

# Thank you for listening!

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