GARD – Genomic Allergen Rapid Detection

Validation, Predictive Performance and Applicability for Cosmetic Substances

Andy Forreryd, PhD, Scientist

SenzaGen develops **in vitro assays** for **immunotoxicological endpoints** using **genomics** technology.

Based in **Lund**, **Sweden**. The company was formed in 2010 as a spinout from **Lund University**. SenzaGen currently employs 20 people.

Our lead product, **GARD – Genomic Allergen Rapid Detection**, is a state of the art platform for assessment of **chemical sensitizers**.

The **challenge:** to find the perfect in vitro **safety assessment**

Predictive performance

Simple workflow

In vitro

SENZA GEN

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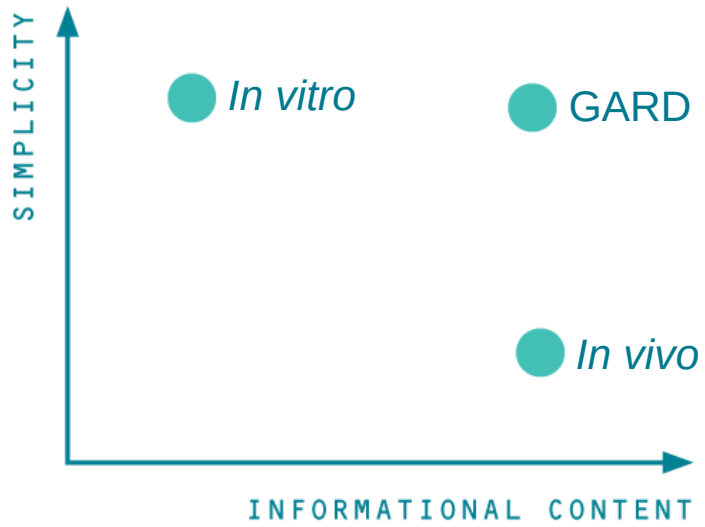

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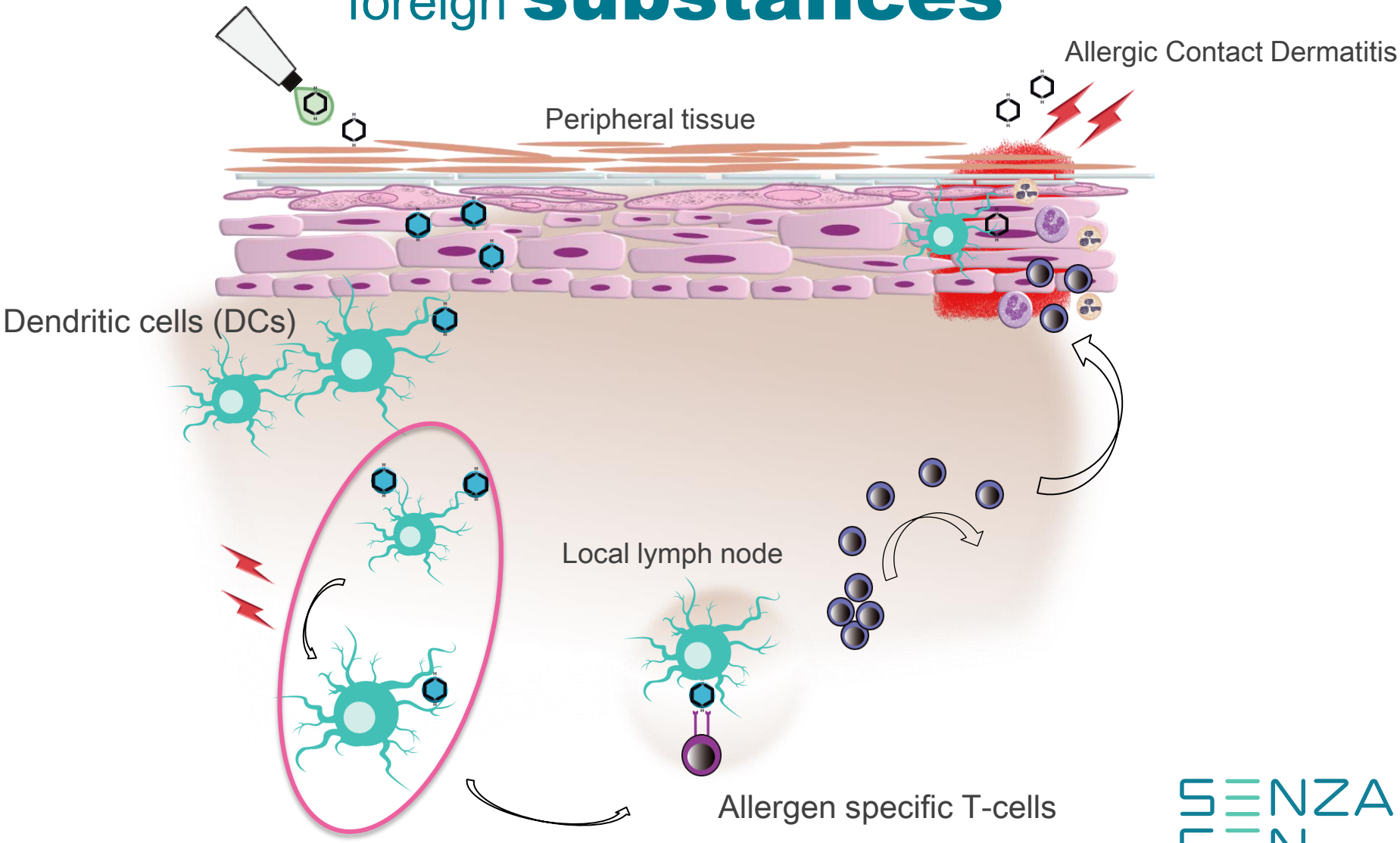
An intricate **response** to foreign **substances**



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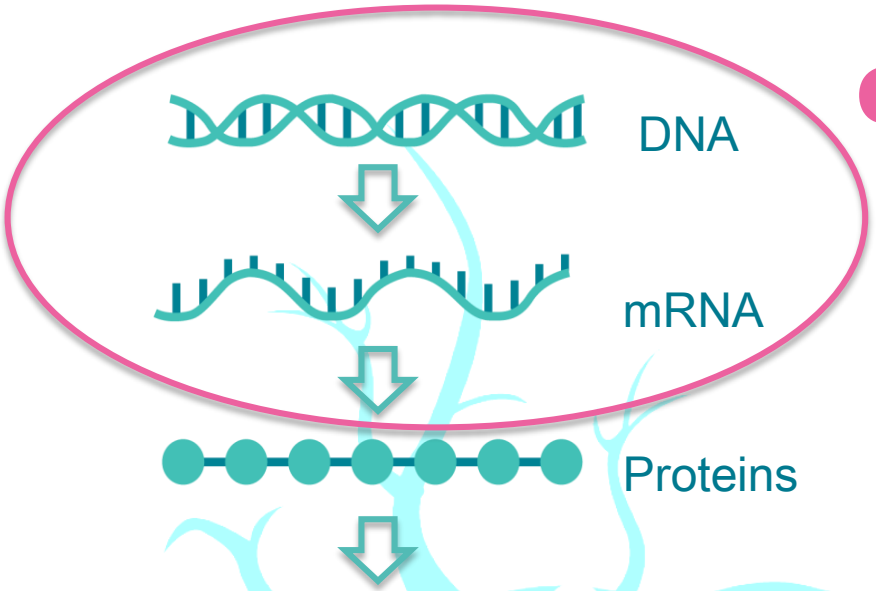


Genomics

Recognition of foreign substances.

Activation of self-defence mechanisms.

Cellular stress responses.

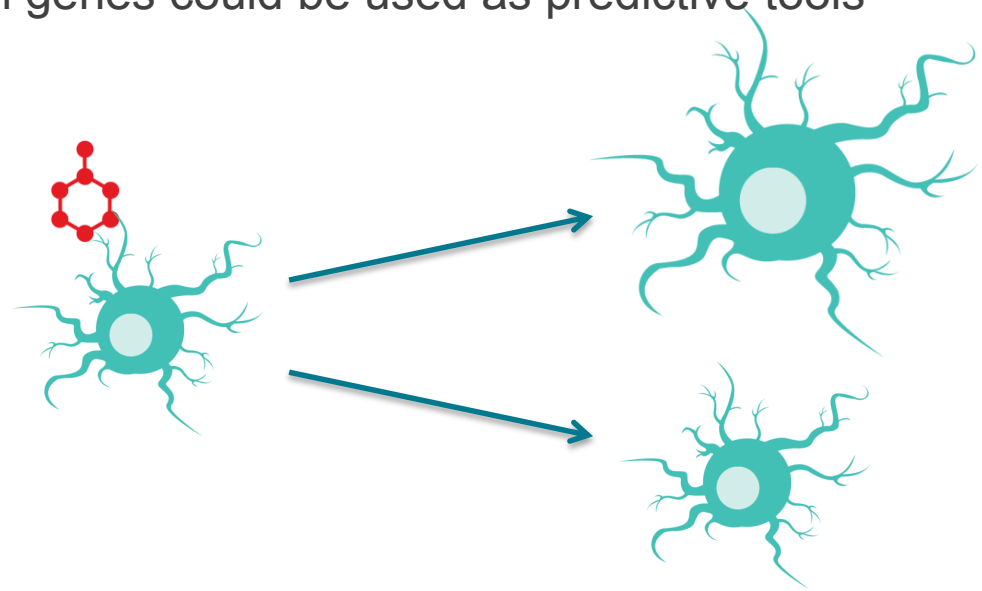
Communication with cells of the immune system.

The **GARD** method **mimics** the **immune response**

Assay Development

- There should exist genes and pathways in DCs that are differentially expressed depending on the stimuli

- Such genes could be used as predictive tools



Sensitizer

Non-sensitizer

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The **GARD** method

mimics the **immune response**

Assay development – The training set

SenzaCells, an *in vitro* model for Dendritic Cells were stimulated with a reference panel of

20 **Sensitizers**
20 **Non-sensitizers**

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	

BMC Genomics

Johansson *et al. BMC Genomics* 2011, **12**:399
http://www.biomedcentral.com/1471-2164/12/399

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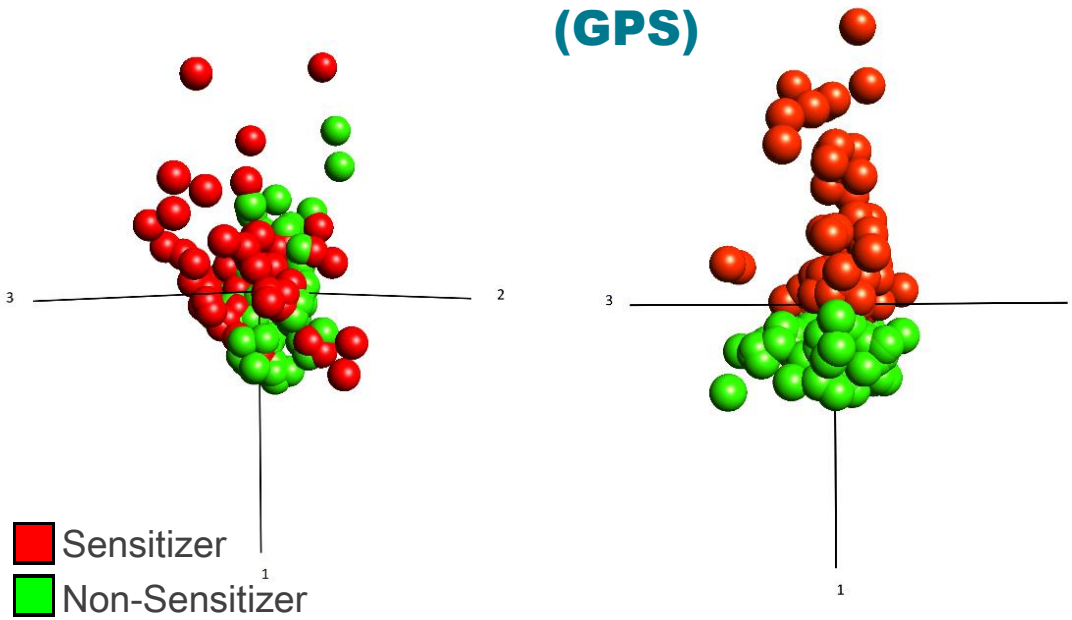
The **GARD** method

mimics the **immune response**

GARD prediction signature (GPS)



Recognition of foreign substances
e.g. **TLR**s, **RXR**, **AHR**

Self-defence mechanisms
e.g. **CD80**, **CD86**

Cellular stress responses
e.g. **NRF2**-pathway

Communication
e.g. chemotaxis receptors

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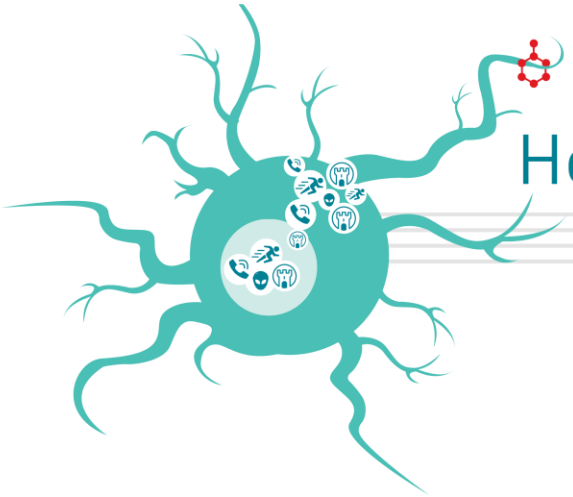
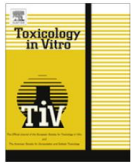


How to **GARD**™ your product in six steps



The GARD assay for assessment of chemical skin sensitizers

Henrik Johansson, Ann-Sofie Albrekt, Carl A.K. Borrebaeck, Malin Lindstedt*

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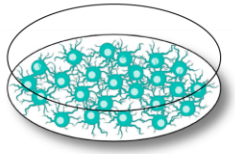

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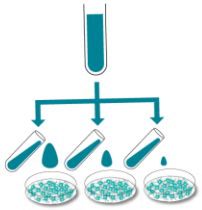

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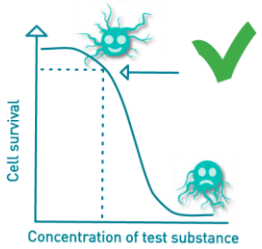

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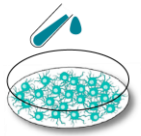

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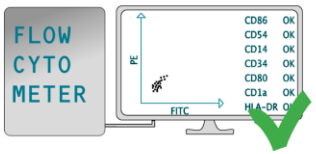

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Extract RNA from the cells

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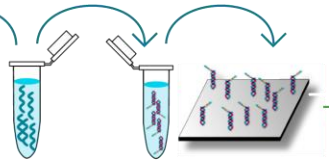

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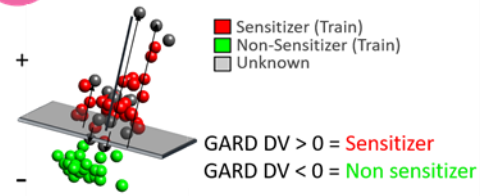

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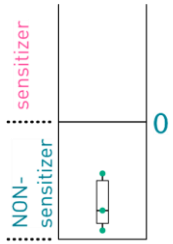

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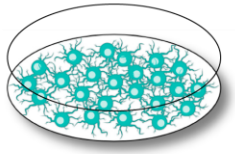

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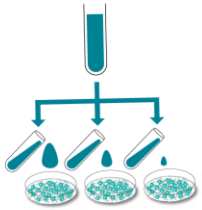

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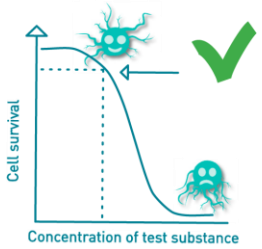

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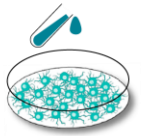

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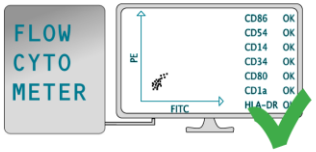

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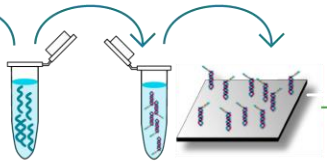

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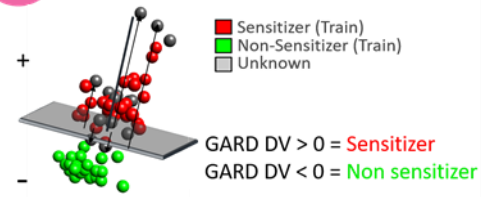

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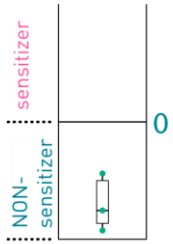

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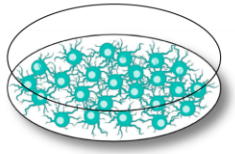

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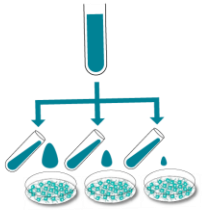

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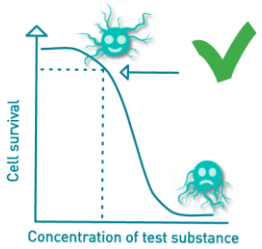

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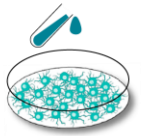

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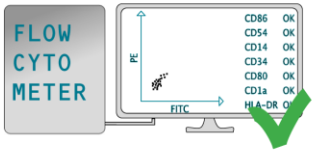

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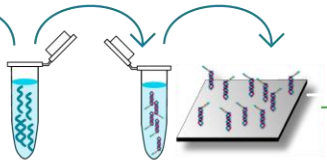

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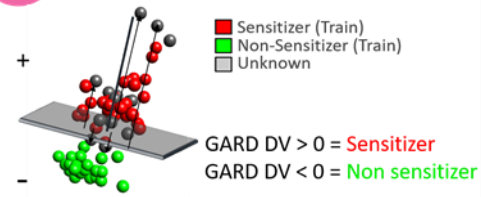

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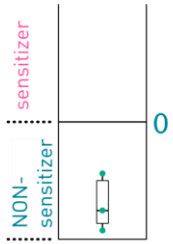

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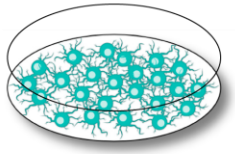

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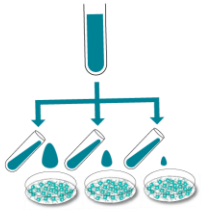

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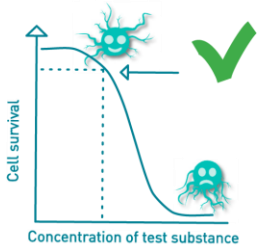

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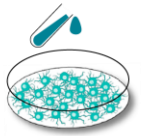

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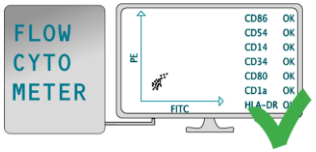

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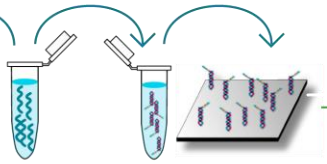

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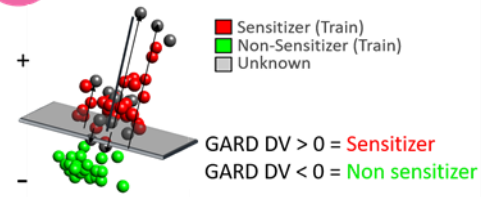

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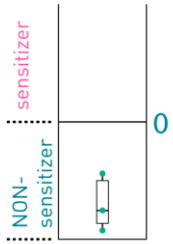

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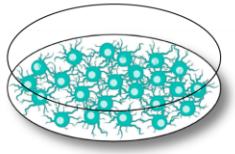

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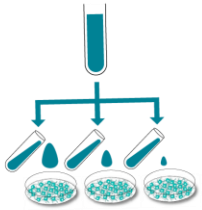

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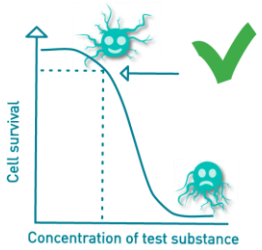

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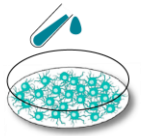

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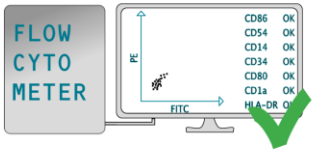

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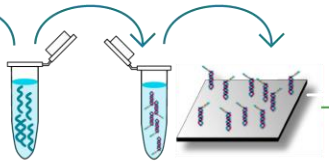

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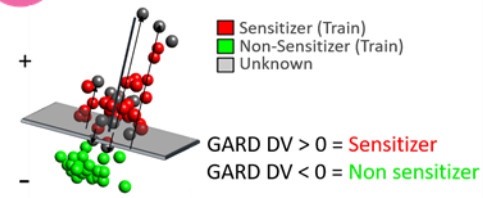
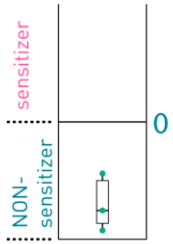
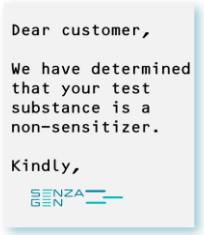


Upload the results to the GDAA web app. One press of the button and the algorithm crunches the data

6 Results





The results are yours!

in-house

in-house

in-house or

in-house

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The GARD method - in-house validation & Performance

Dataset	Sensitivity	Specificity	Accuracy	Reference
GARD in-house validation	89% (17/19)	86% (6/7)	88% (23/26)	Johansson, 2014
Technology transfer and method optimization	94% (16/17)	83% (10/12)	90% (26/29)	Forreryd, 2016
Cosmetic Europé Dataset	93% (50/54)	56% (10/18)	83% (60/72)	Johansson, 2017
Accumulated performance	92% (83/90)	70% (26/37)	**86% (109/127)**	-

Genomic Allergen Rapid Detection In-House Validation—A Proof of Concept

Henrik Johansson,[*] Frida Rydnert,[*] Jochen Kühnl,[†] Andreas Schepky,[†] Carl Borrebaeck,[*] and Malin Lindstedt[*,1]

From genome-wide arrays to tailor-made biomarker readout – Progress towards routine analysis of skin sensitizing chemicals with GARD

Andy Forreryd [a], Kathrin S. Zeller [a], Tim Lindberg [a], Henrik Johansson [b], Malin Lindstedt [a,*]

Evaluation of the GARD assay in a blind Cosmetics Europe study

Henrik Johansson[1], Robin Gradin[1], Andy Forreryd[2], Maria Agemark[1], Kathrin Zeller[2], Angelica Johansson[1], Olivia Larne[1], Erwin van Vlier[3], Carl Borrebaeck[2] and Malin Lindstedt[2]

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The **GARD** method - in-house **validation** & **Performance**

	DPRA (TG 442C)	ARE-NRF2 (TG 442D)	h-CLAT (TG 442E)	2 out of 3 ITS	GARD (TGP 4.106)
Accuracy	80%	83%	77%	83%	**88%**
Sensitivity	78%	84%	80%	84%	**90%**
Specificity	**83%**	78%	67%	78%	**83%**

Based on 69 overlapping compounds from Asturiol et. al., Toxicology in vitro, 2016.

Roberts, D.W. John Moore University, Liverpool. Regul Toxicol Pharmacol, 2018.

Is a combination of assays really needed for non-animal prediction of skin sensitization potential? Performance of the GARD™ (Genomic Allergen Rapid Detection) assay in comparison with OECD guideline assays alone and in combination.

Roberts DW[1].

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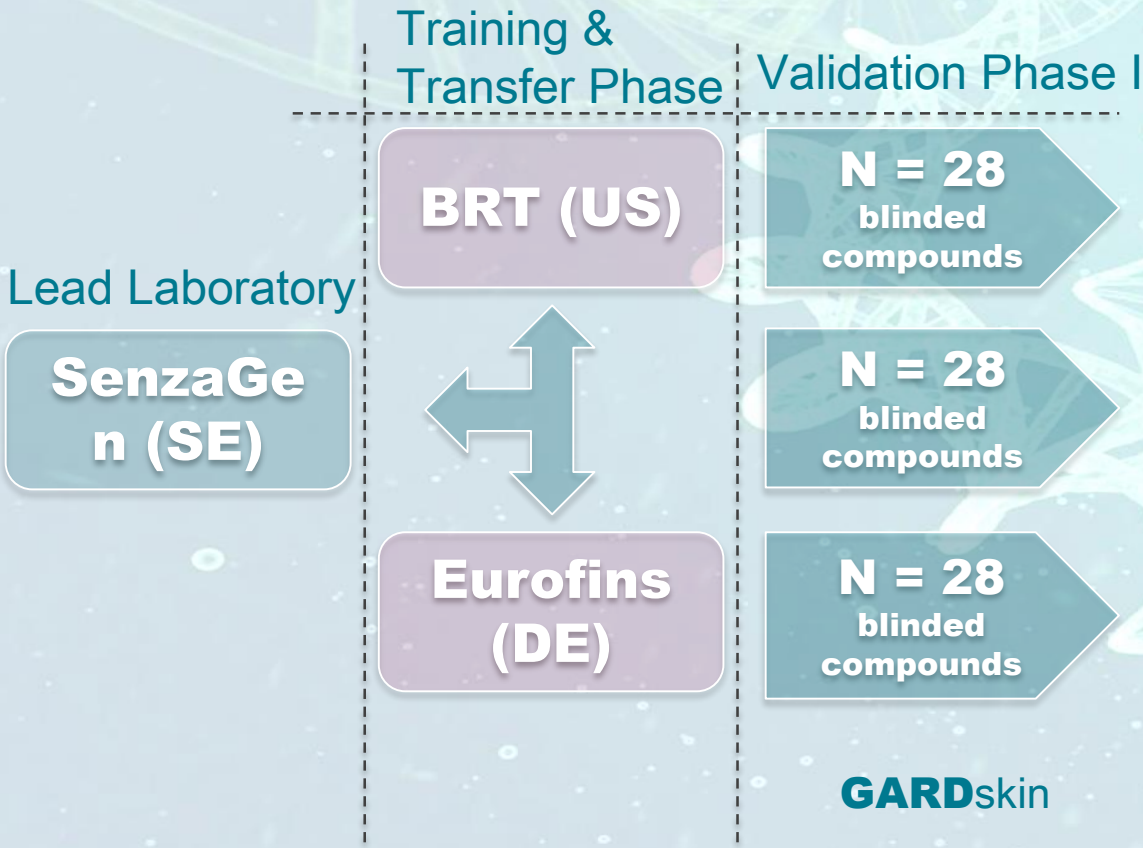
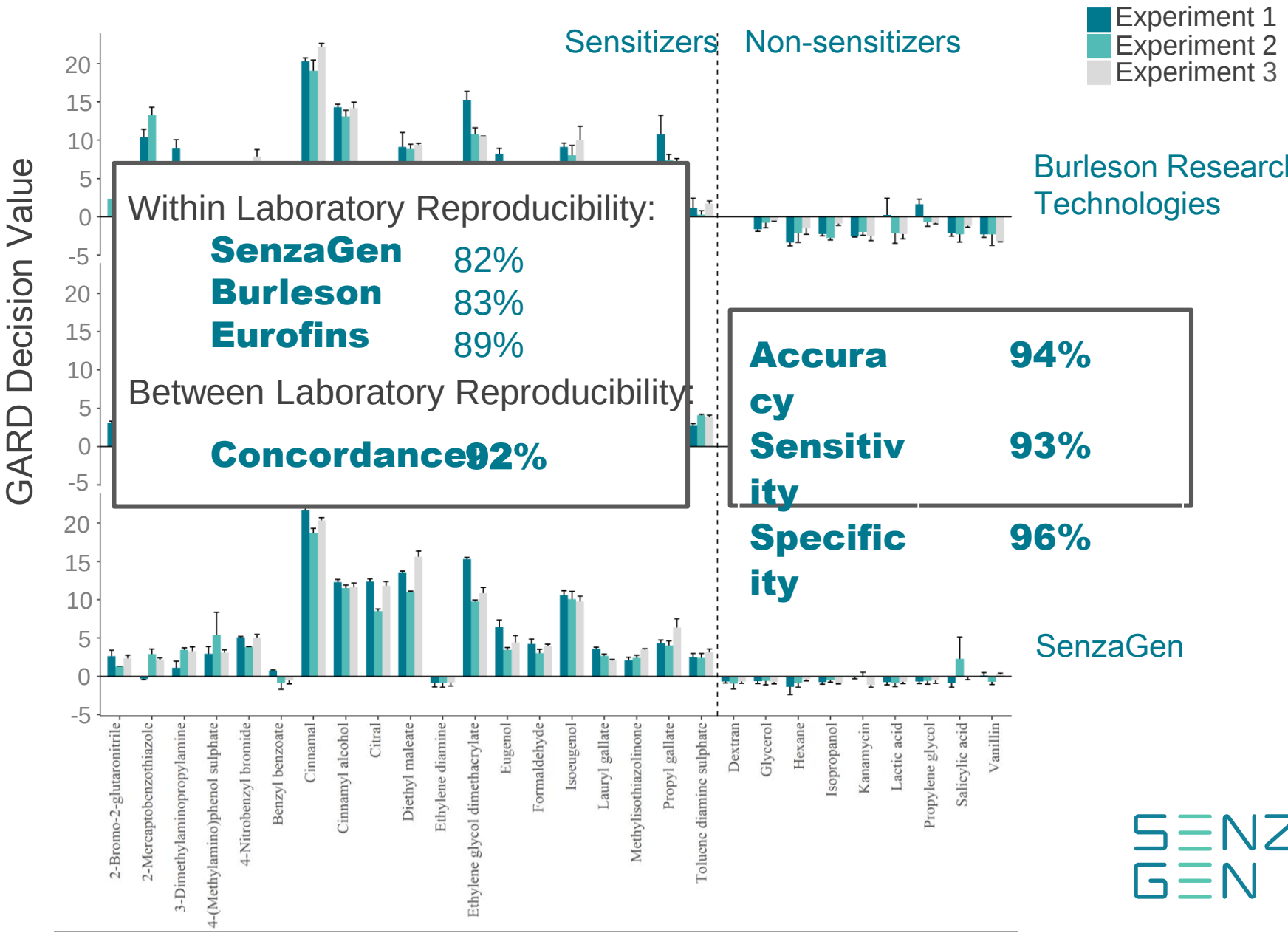
GARDskin validation



GARDskin validation – Validation Phase I



Within Laboratory Reproducibility:

SenzaGen	82%
Burleson	83%
Eurofins	89%

Between Laboratory Reproducibility:

Concordance	**92%**

Burleson Research Technologies

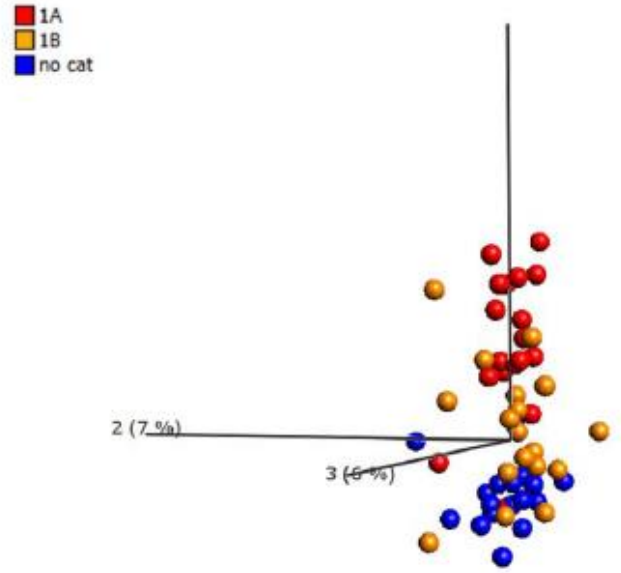
Accuracy	**94%**
Sensitivity	**93%**
Specificity	**96%**

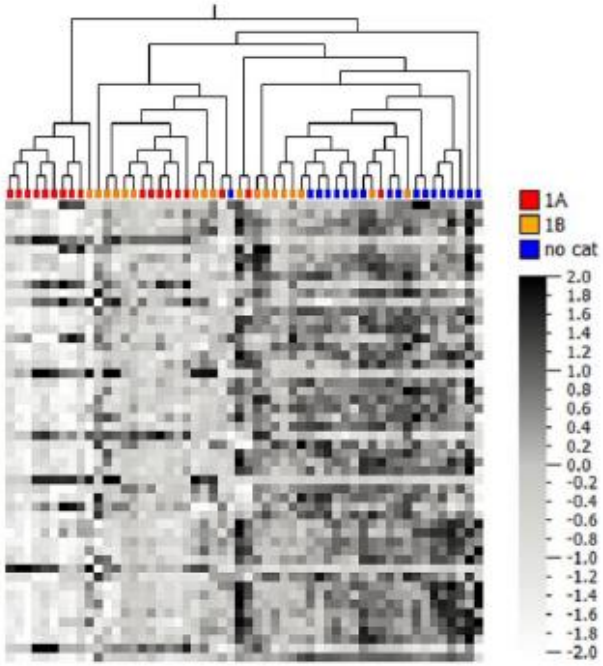
SenzaGen

SENZA GEN

GARDpotency – additional analysis of **complementary** biomarker signature

GARDpotency prediction signature





The GARD platform for potency assessment of skin sensitizing chemicals

Kathrin S. Zeller[1], Andy Forreryd[1], Tim Lindberg[1], Robin Gradin[1,2], Aakash Chawade[3] and Malin Lindstedt[1]

ALTEX
ALTERNATIVES TO ANIMAL EXPERIMENTATION

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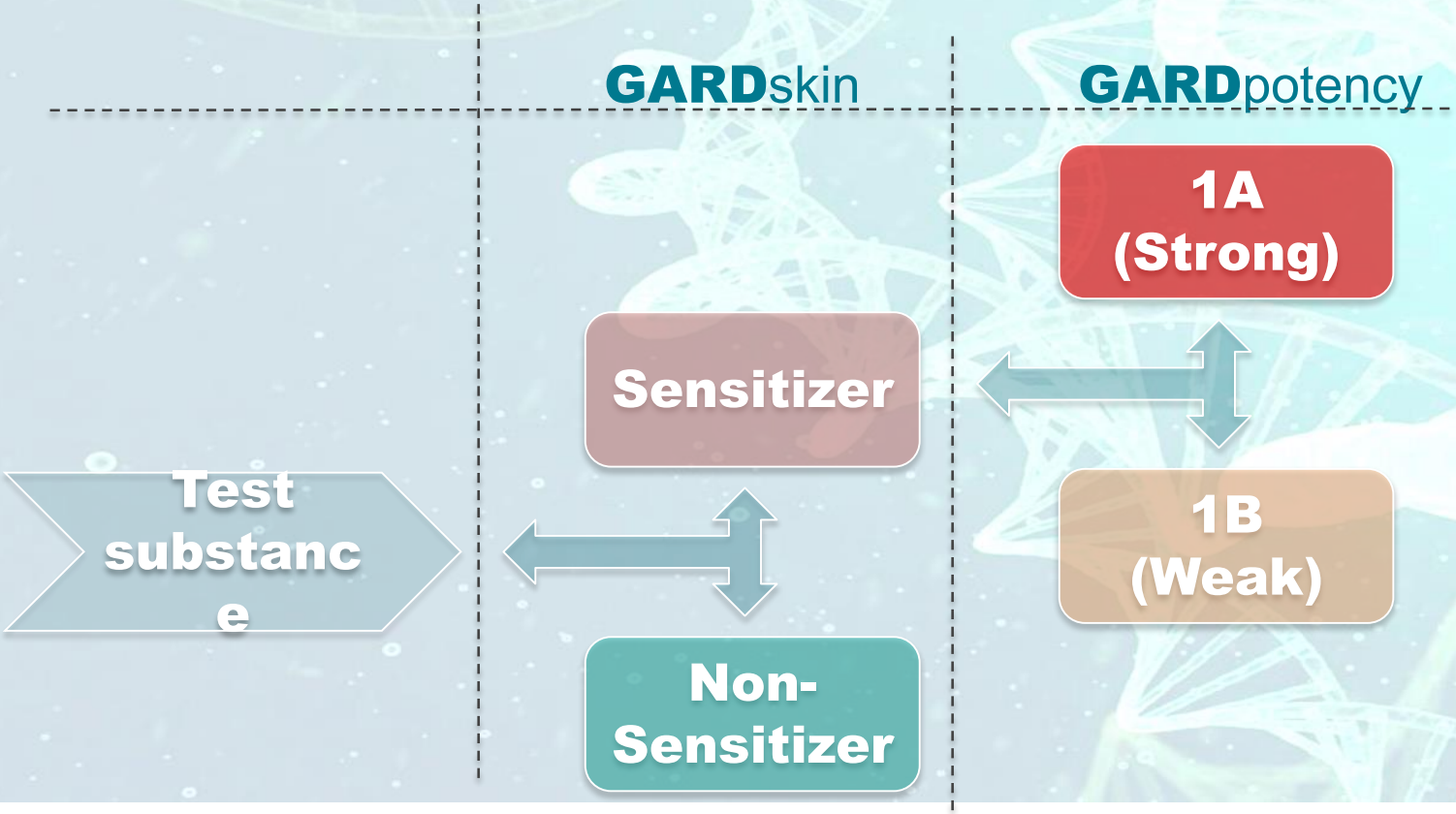
GARDskin & GARDpotency - complete

Assessment in a Tiered Approach



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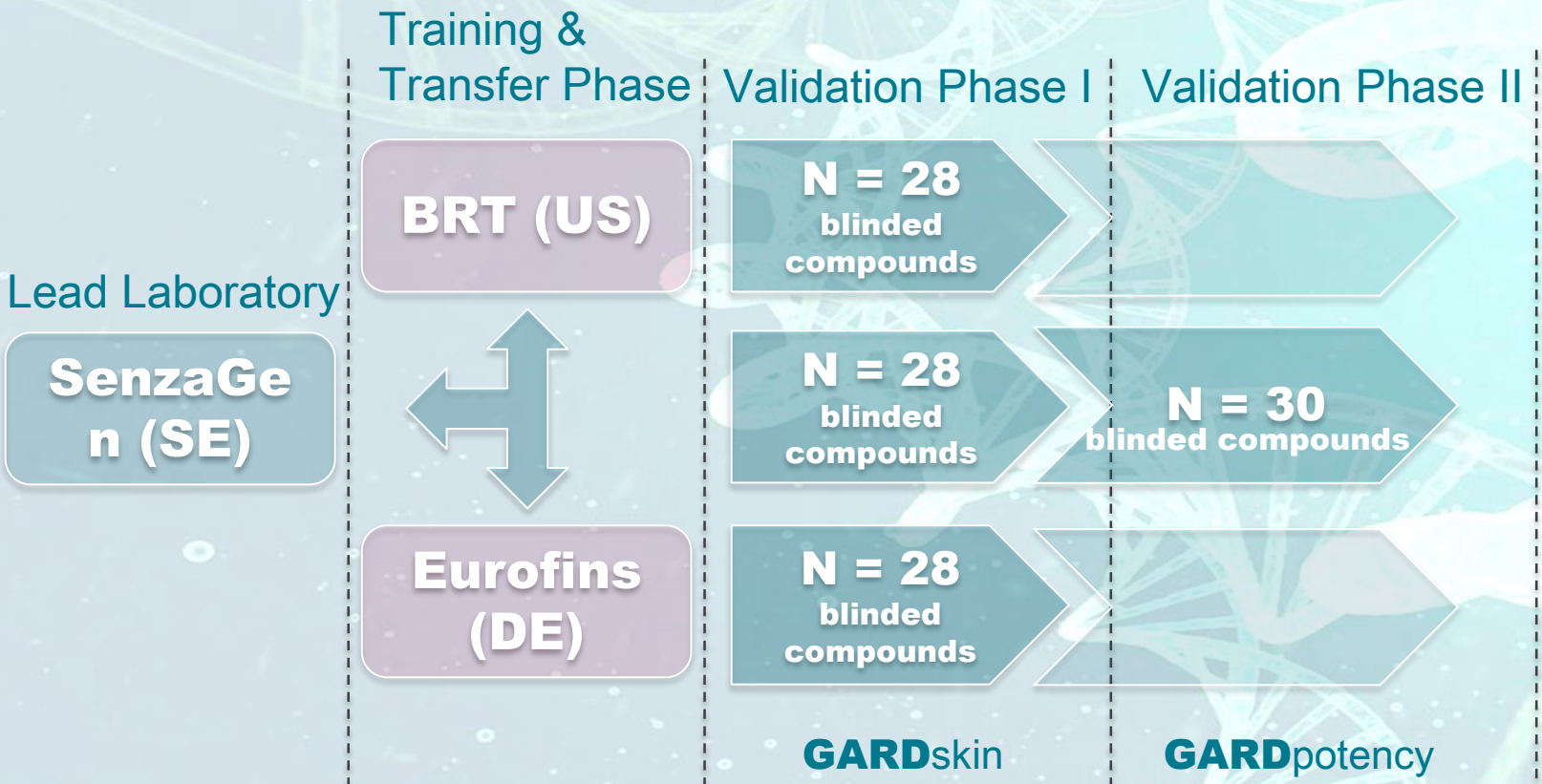
GARDpotency validation – Validation phase II



Accuracy:

83%

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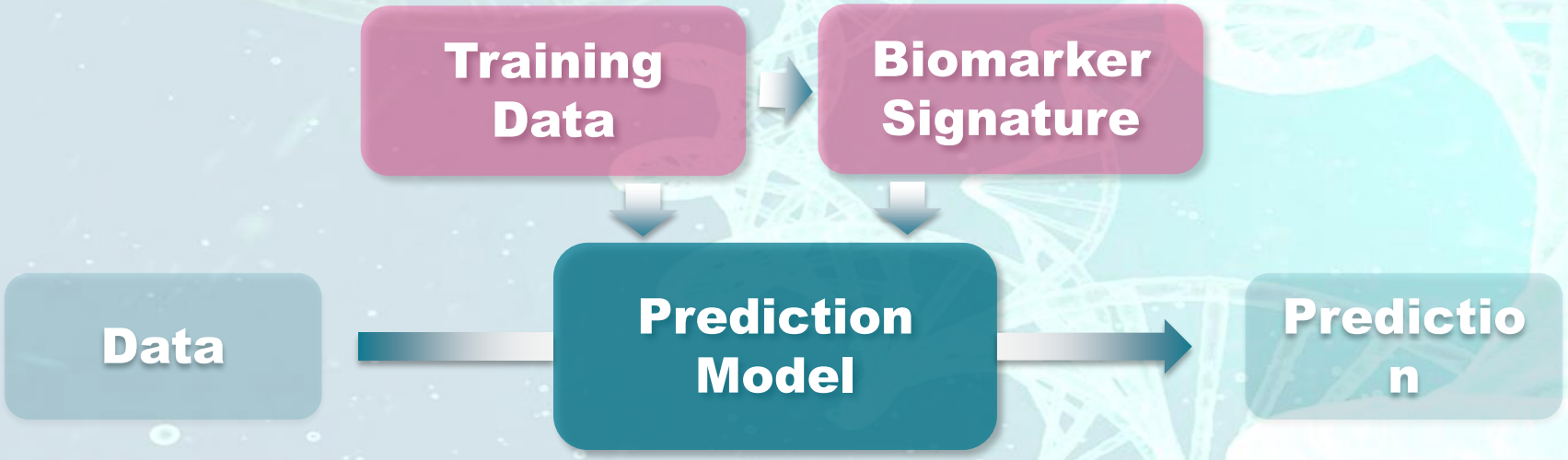
GARD applications – GARDair & GARD for protein



Gene Expression Patterns in exposed cells

Pattern Recognition Algorithms

Biological endpoint

HORIZON 2020
The EU Framework Program for Research and Innovation

European Commission

This project has recieved funding from the European Union´s *Horizon 2020 research and innovation program* under grant agreement No 756014.

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GARD – **G**enomic **A**llergen **R**apid **D**etection

GARD outperforms OECD guideline approaches and reports the **highest predictive performance** for skin sensitization.

GARD enables potency assessment according to CLP with an outstanding **predictive performance**.

Versatile test systems allows for a **broad applicability domain**.

The technology is readily **available today**!

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Lund University

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Tim Lindberg
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Dr. Ann-Sofie Albrekt
Dr. Aakash Chawade

3RsMC

Erwin Roggen, validation manager

Thank you for listening!