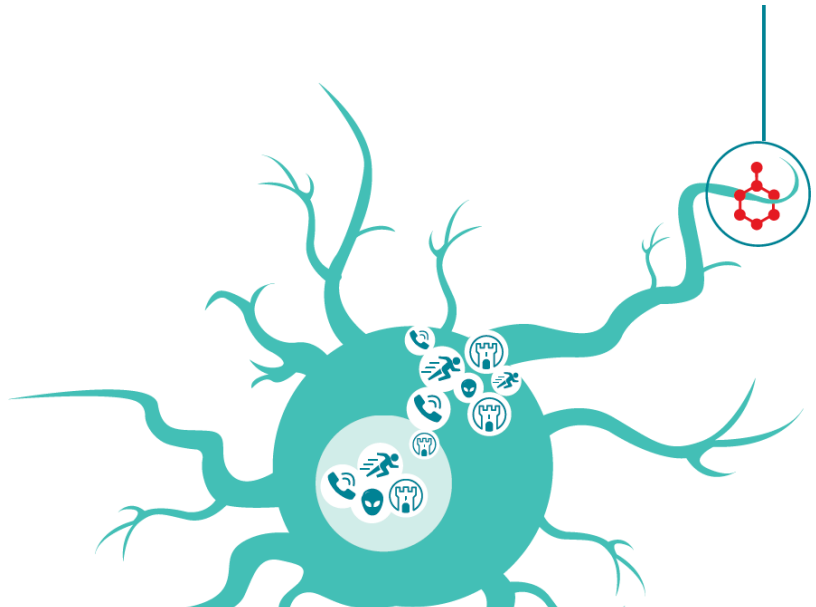
All you need to know about GARD™

In vitro skin and respiratory sensitization testing
based on genomics and machine learning

Feburary 25th, 2020



SenzaGen in short

- Founded: 2010. Operational: 2014.
- Spin-out from Lund University. Research in immunotechnology.
- HQ and Lab in Lund. Sales office in US. Highly multidisciplinary team.
- Listed on Nasdaq First North, Stockholm in 2017.

The GARD™ PLATFORM

State-of-the-art test platform for assessment of chemical sensitizers offering:

- High performance
- Broad applicability
- Efficiency

ASSAYS

- GARD™skin
- GARD™potency
- GARD™skin Medical Device
- GARD™air

PRODUCT DEVELOPMENT

Robust technology platform with large potential within various toxicological applications and markets.

BUSINESS MODEL

- Sales via license CROs and distributors, and via the HQ in Sweden and a US sales office.
- Laboratory and Product Development is at HQ.

OUR VISION

Replace animal experiments
Establish a new industry standard

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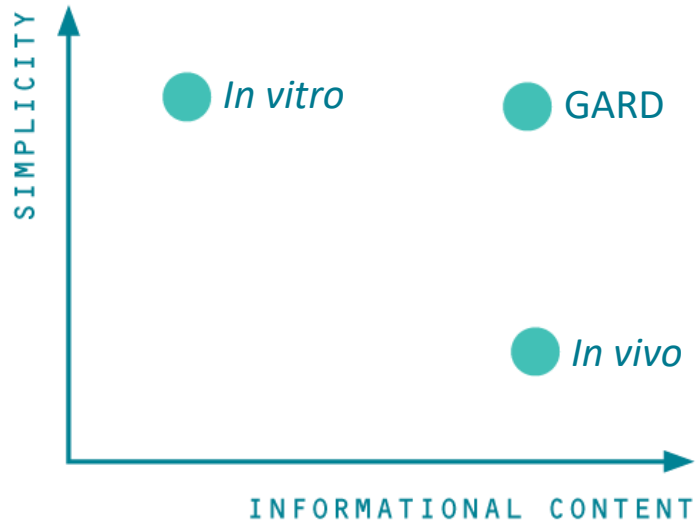
Predictive toxicology: a scientific field in change

Predictive performance

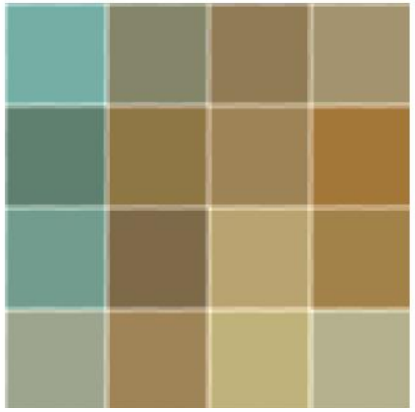
Simple workflow

In vitro

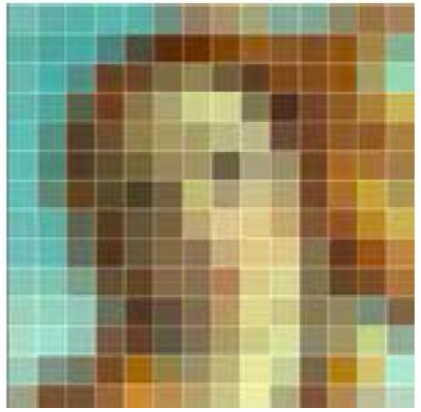


High informational content → Closer to reality

AN IN VITRO MODEL, WHERE FEW POINTS ARE MEASURED, GIVES NON-CONCLUSIVE RESULTS.



BY MEASURING A LARGE NUMBER OF PARAMETERS, THIS MODEL MIMICS REALITY AND GIVES CONCLUSIVE RESULTS.



Increased number of biomarkers

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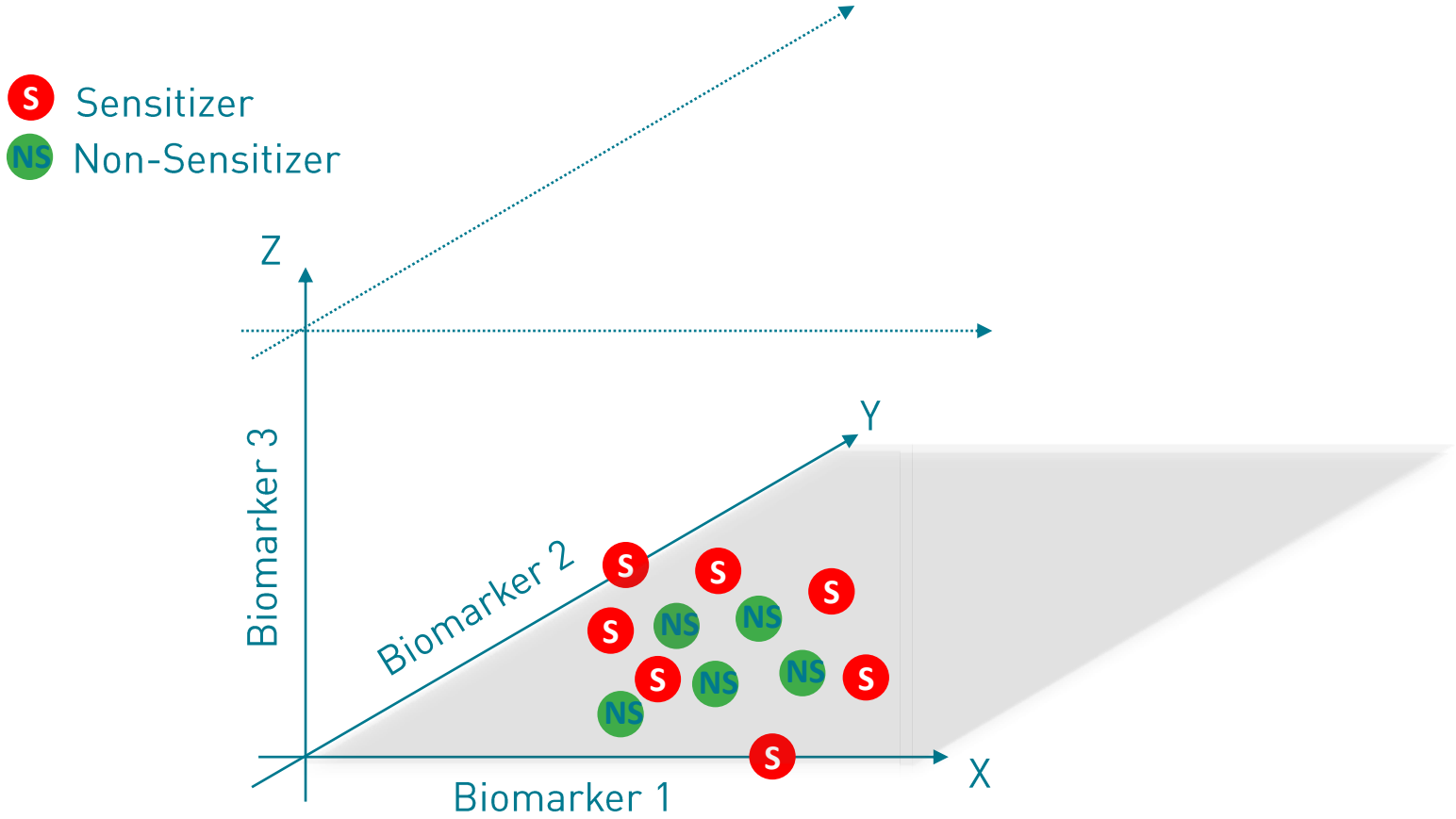
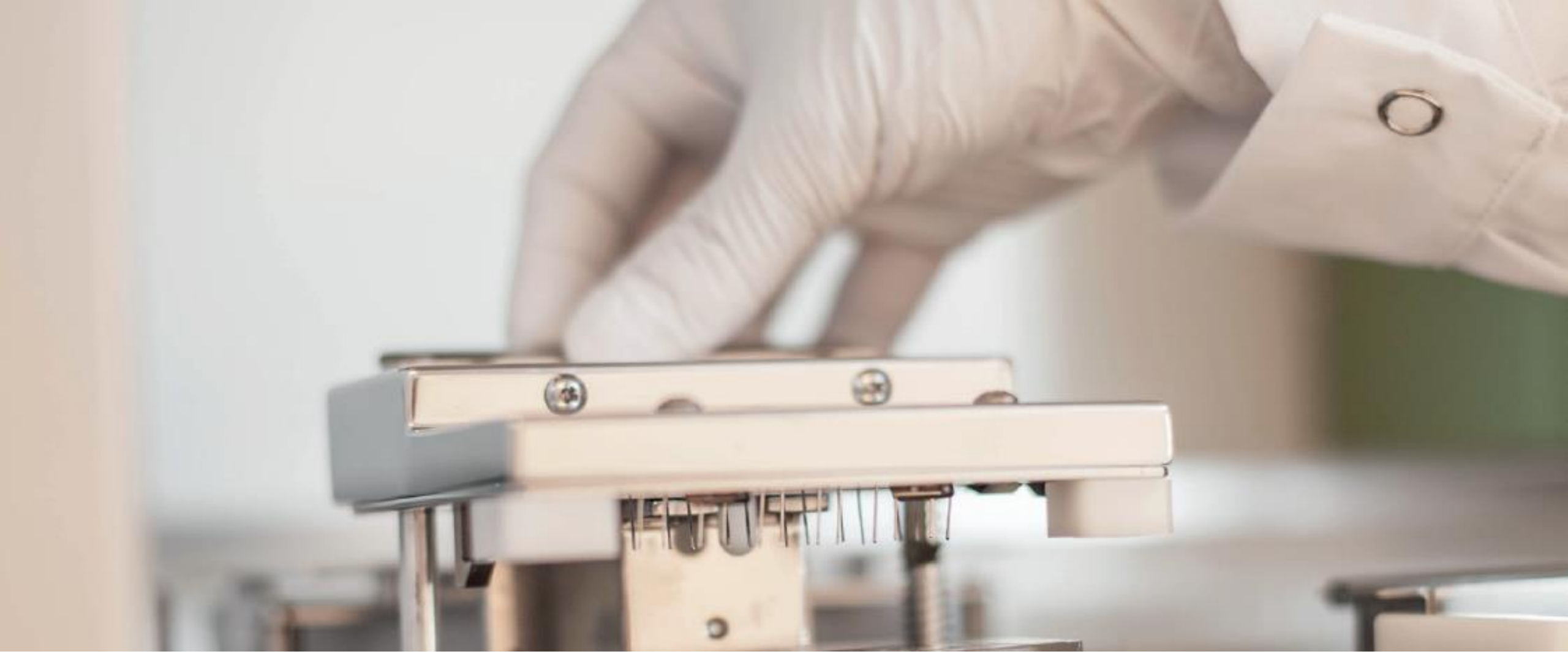
High informational content → Closer to reality

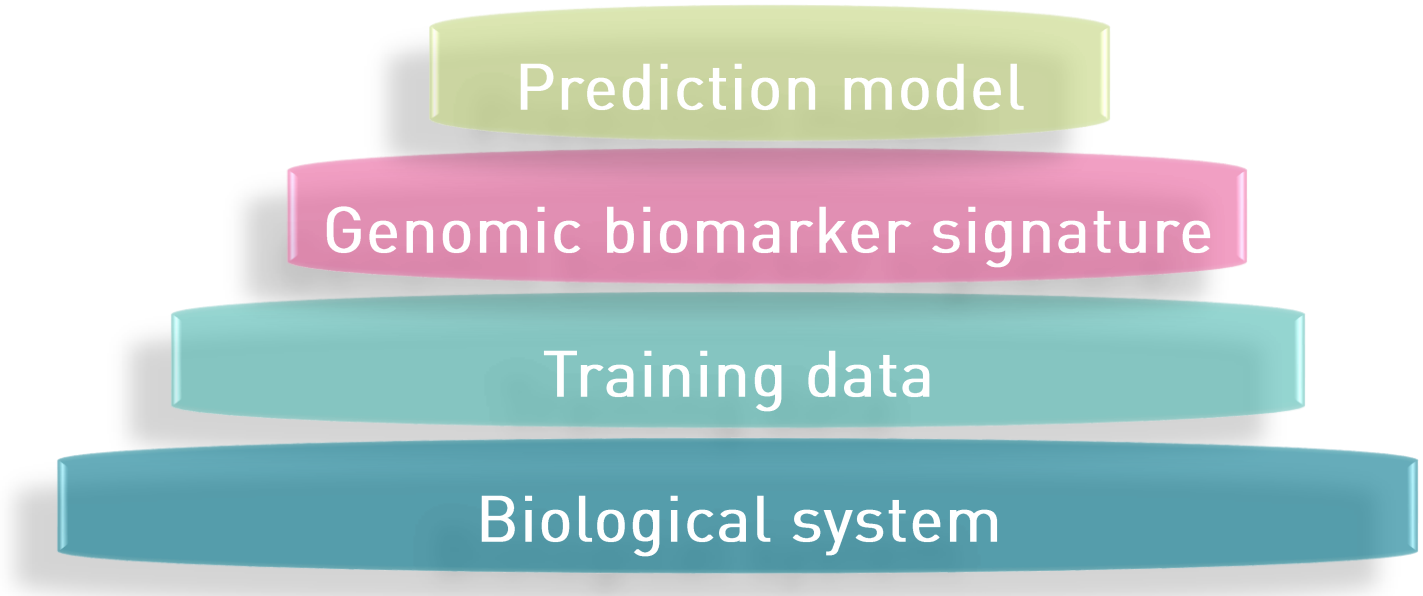




The GARD™ technology platform – Assay development

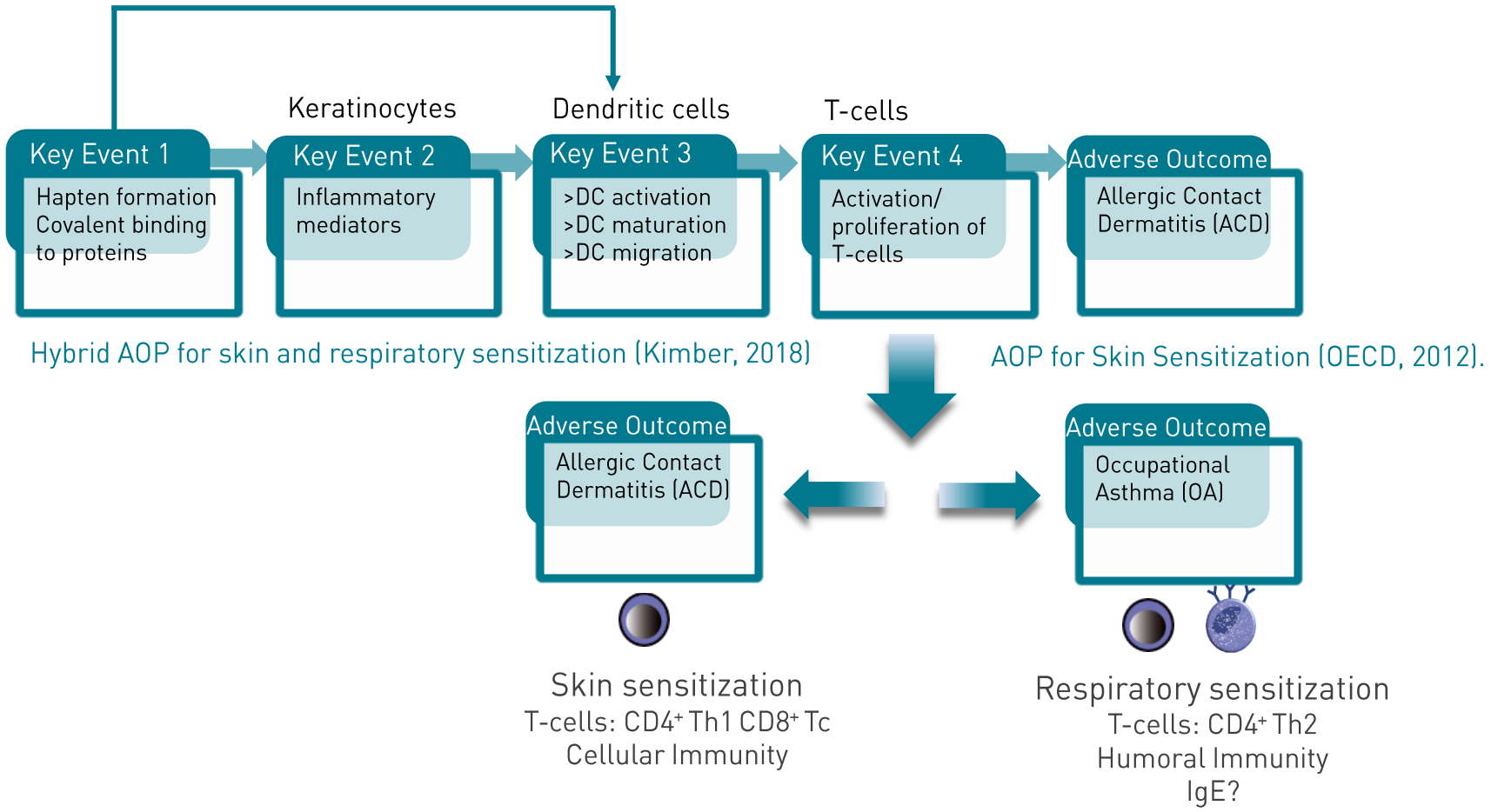
Human relevant cells in combination with Genomics and machine learning

The GARD platform – how it works

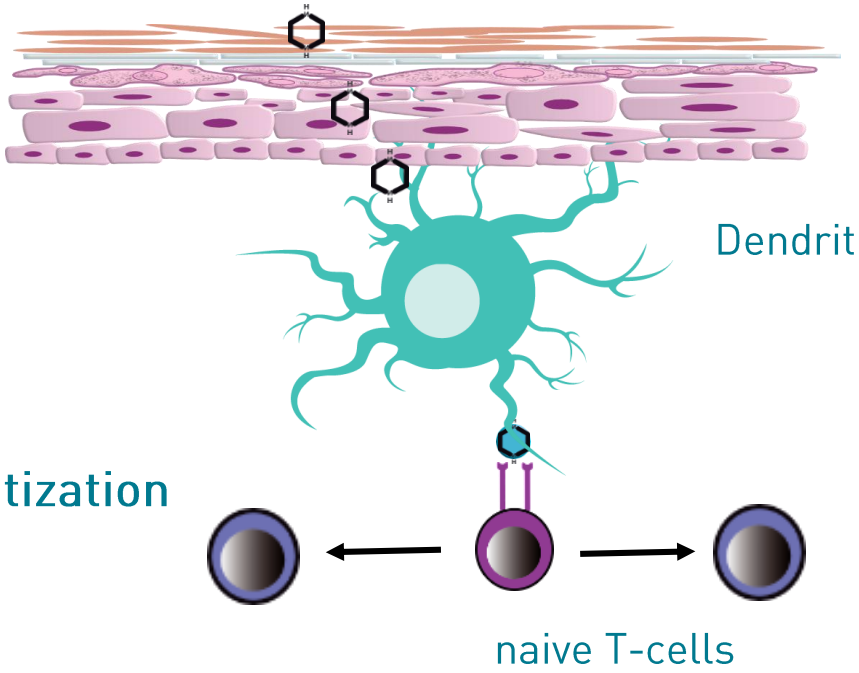


The GARD platform – biological system

Background – the Adverse Outcome Pathway



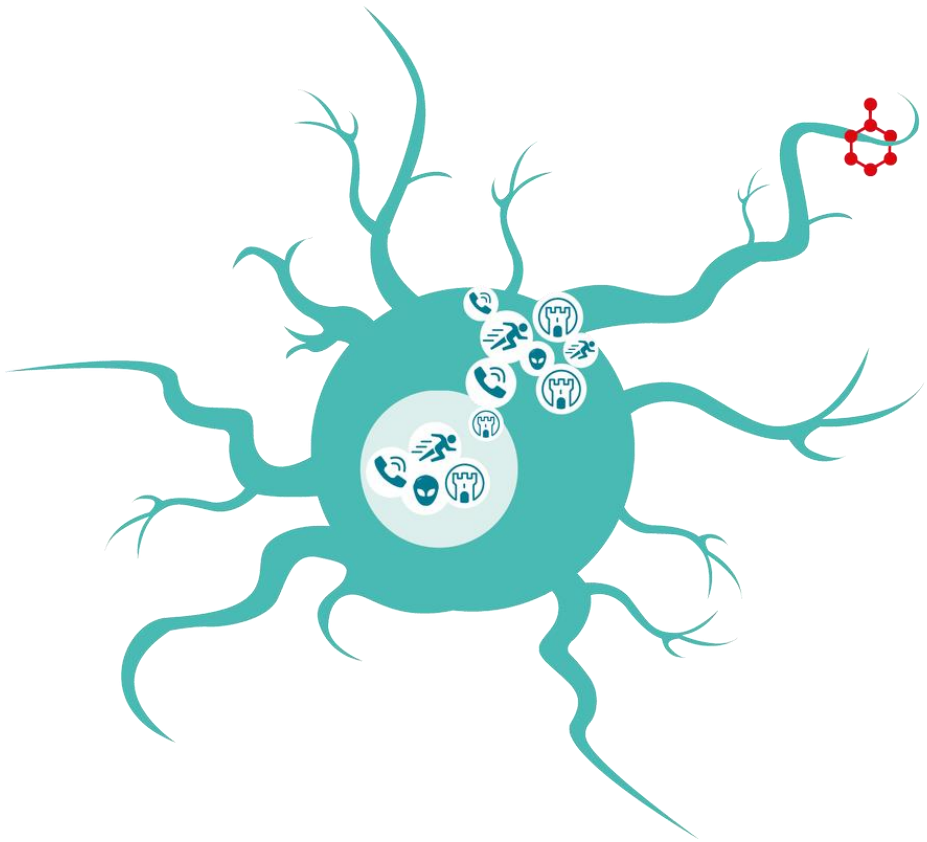
Background– Dendritic cells (DCs)



Respiratory sensitization
T-cells: $CD4^+$ Th2
Humoral Immunity

Skin sensitization
T-cells: $CD4^+$ Th1 & $CD8^+$ Tc
Cellular Immunity

SenzaCell™ : a human DC-like cell line



Antigen presentation: MHC I, MHC II Cd1d

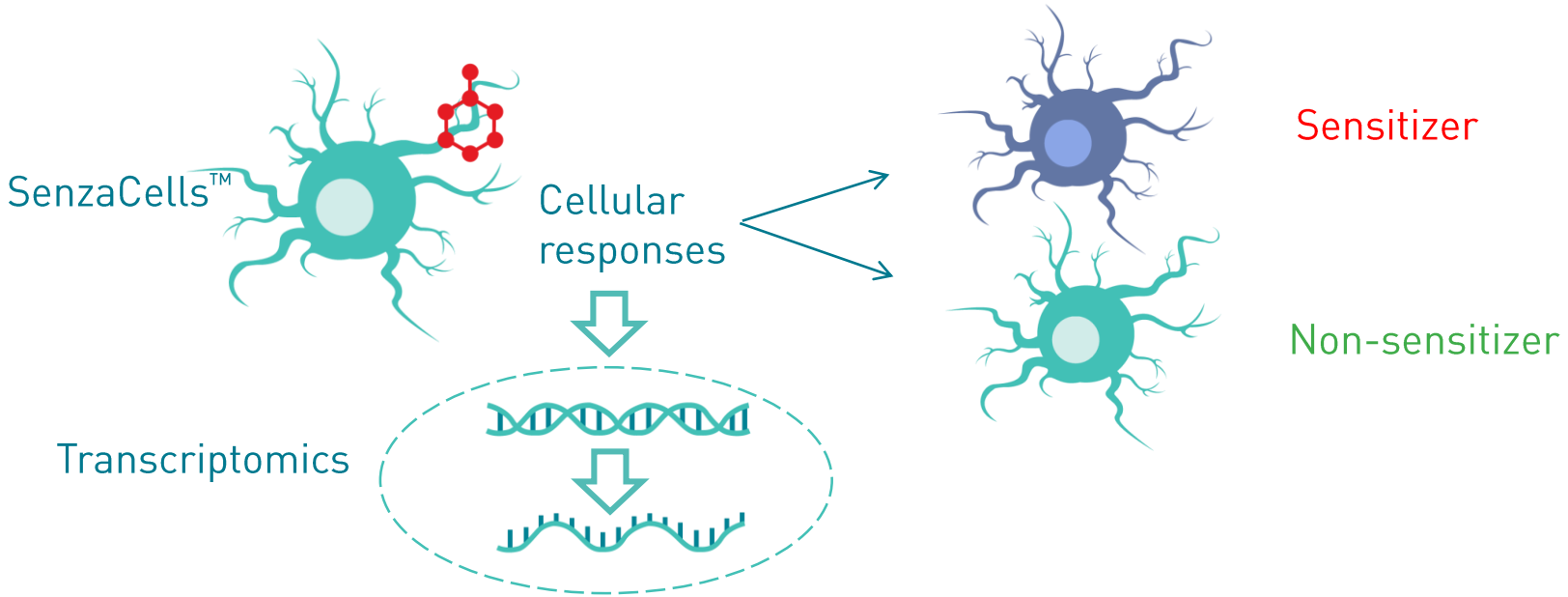
Potent activators of T-cells: CD80, CD86

T-cell polarization: Contains the functional elements for Th_1 & Th_2 polarization

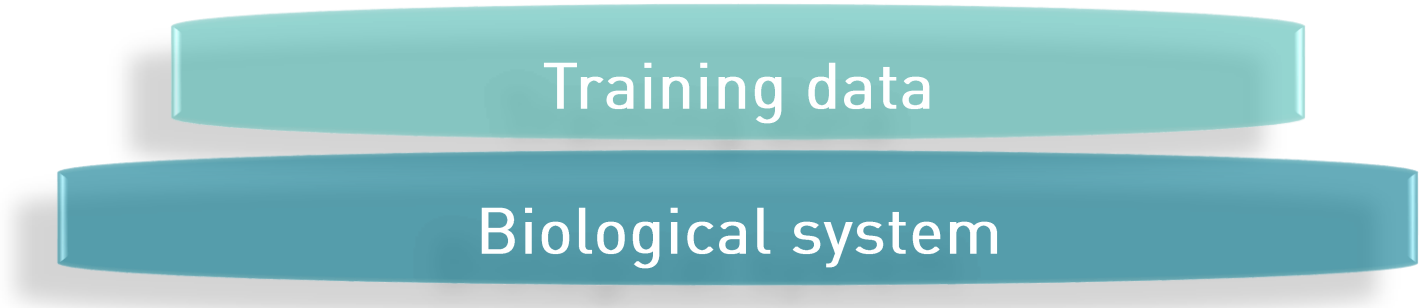
Transcriptomic read-out of the biological response

Assay Development: Hypothesis

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



The GARD platform – Training data



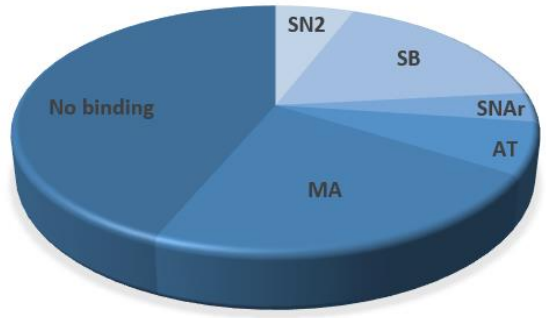
The GARD platform – Training data

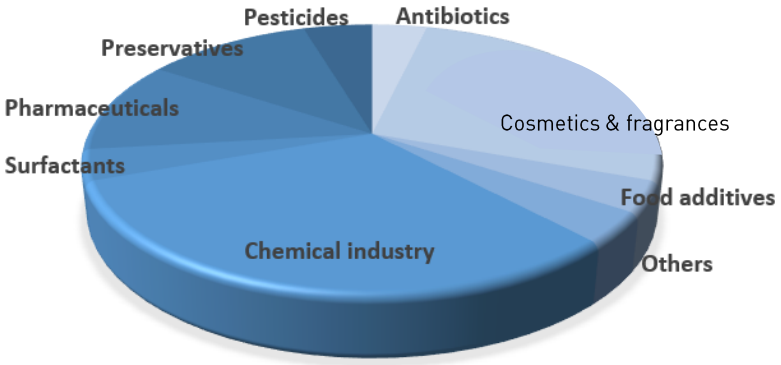
Assay Development:
Defining a Training data set to test the hypothesis.

Training dataset:
A set of well-characterized chemical compounds with known expected outcome.

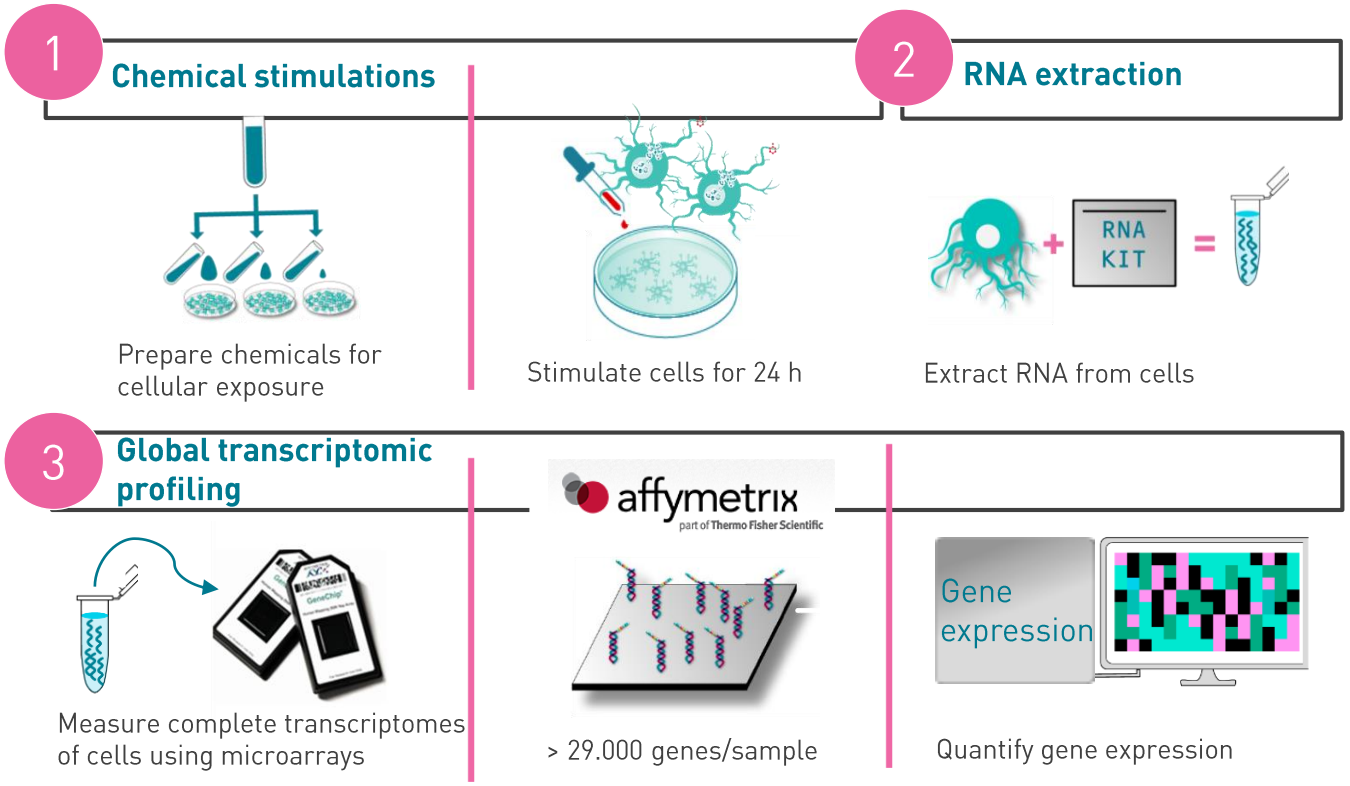
X number of Sensitizers
X number of Non-sensitizers

Ideally, the training set should cover a large chemical space.

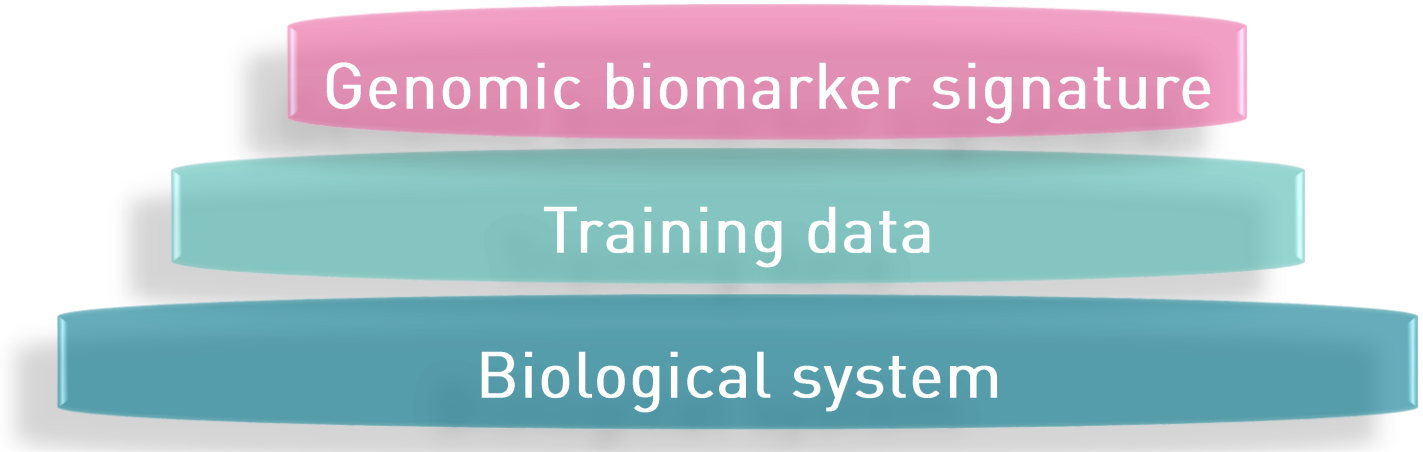




The GARD platform – Training data

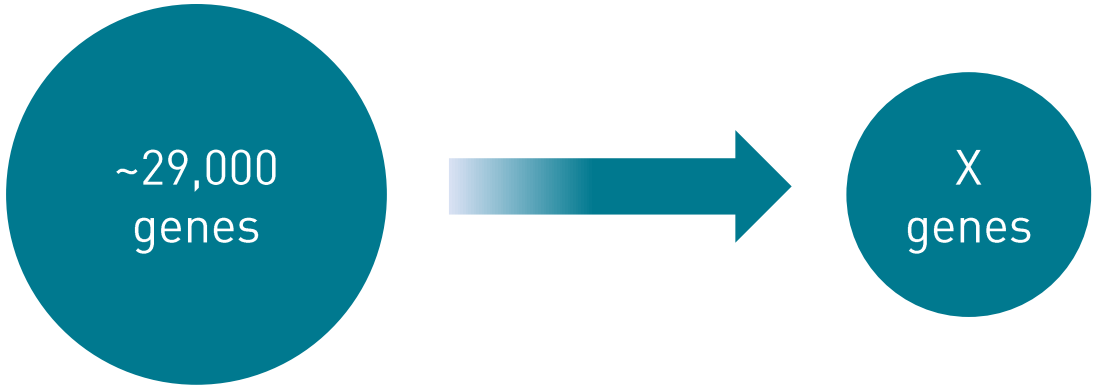


The GARD platform – Genomic biomarker signature

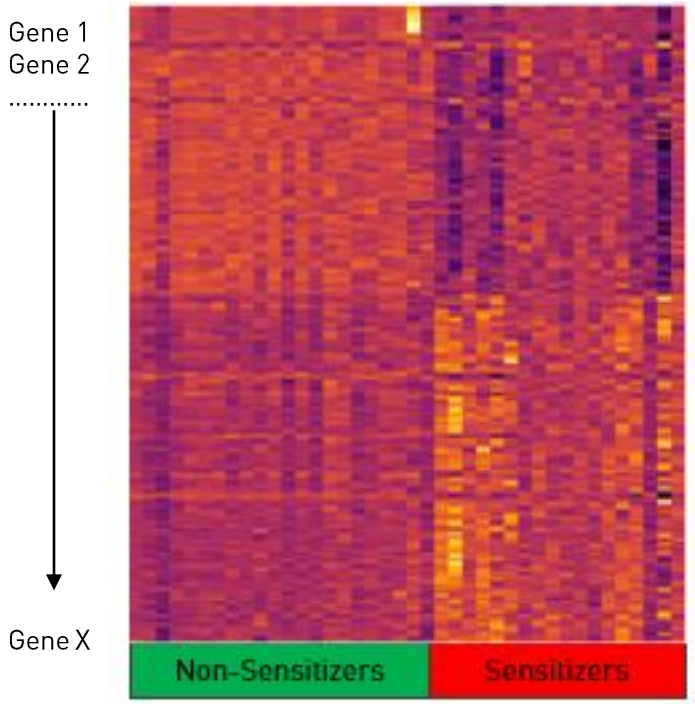


Data-driven biomarker signature identification

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



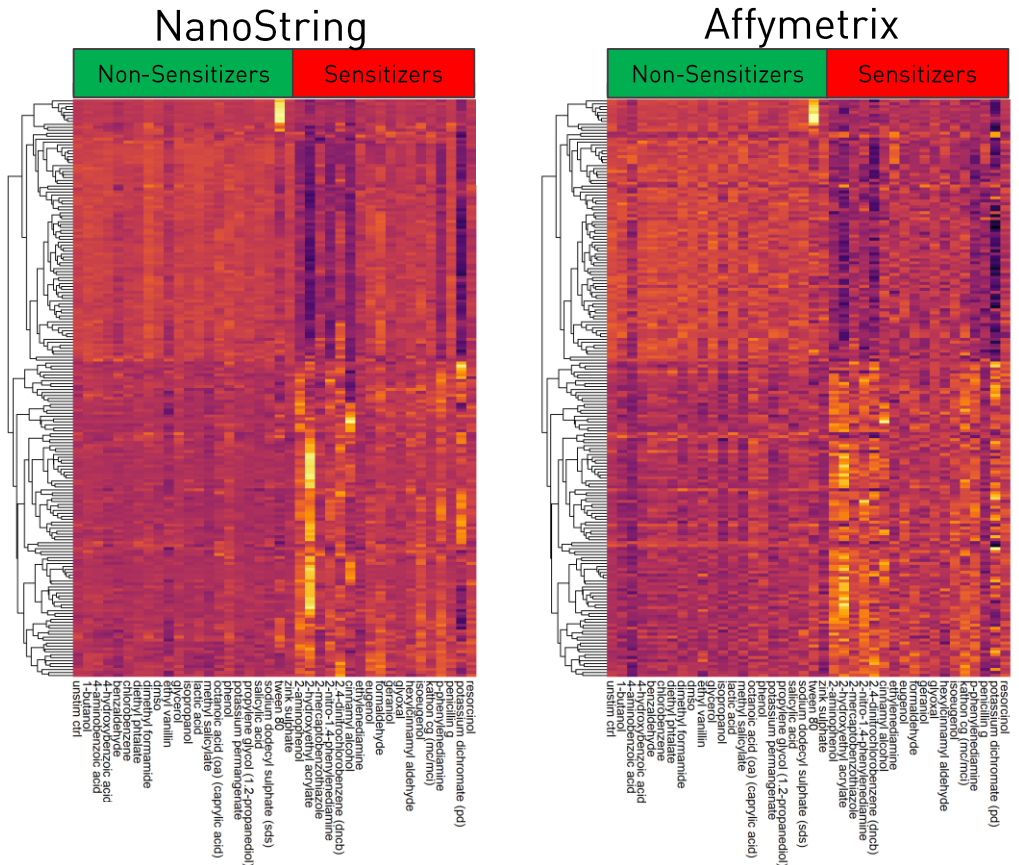
GARD prediction signature



From Affymetrix to NanoString

nanoString

genes: Custom – up to 800 genes
Assay time: 22 h, 15 min hands-on
Throughput: 384 samples/day
Input: Total RNA



affymetrix
part of Thermo Fisher Scientific

genes: > 29.000 genes
Assay time: 4 days
Throughput: 24 samples/batch
Input: cDNA

Spearman correlations for each stimulation between **85-90%**

Gene expression measurement could be reproduced on the independent platform

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

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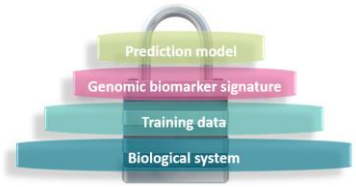
GARD assays: number of biomarker signatures

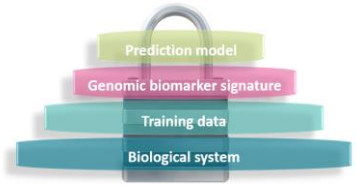
GARD™skin – 200 genes

Skin sensitization testing



GARD™potency – 51 genes

Skin sensitization potency testing according to GHS/CLP



GARD™air – 28 genes

Respiratory sensitization testing

GARD™skin Medical Device – 200 genes

Skin sensitization testing of medical devices

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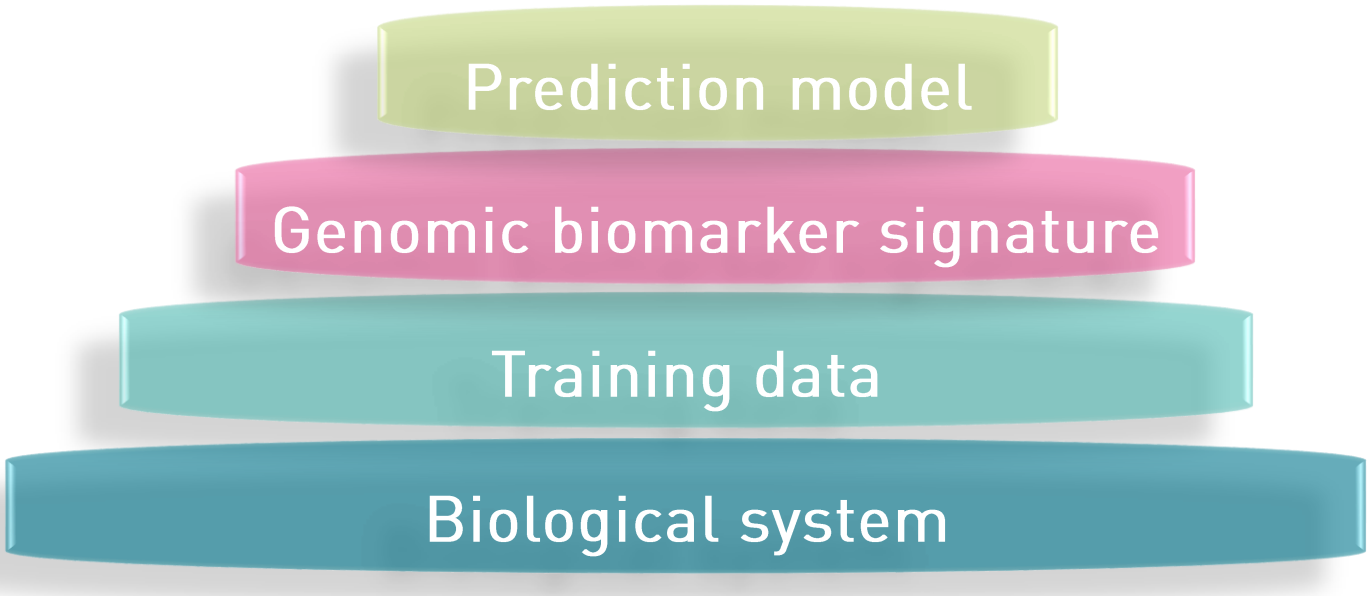
The GARD platform – Prediction model



The GARD platform – Prediction model

What is it?

1

Machine learning is a **computer algorithm** designed to learn from previous experience.

How does it work?

2

By **observing** data from a set of objects ("training data") with known expected outcomes ("labels") .

Training

3

The machine learning algorithm learns & **builds a fixed mathematical model** of the training data.

Class-ification

4

Data from an unlabelled test object is fed into the fixed mathematical model which **assigns a label** to the new data.

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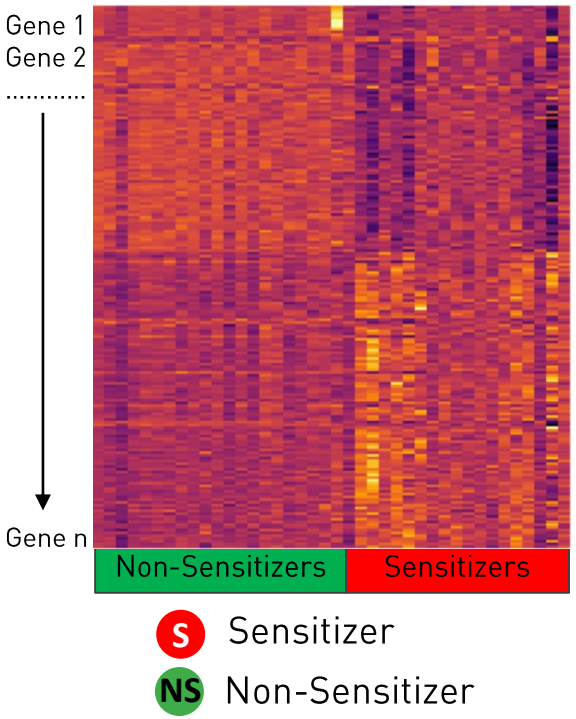
The GARD platform – Prediction model

By **observing** data from a set of objects ("training data") with known expected outcomes ("labels") .

2

How does it work?



The training data:
Gene expression profiles from a set of well-characterized sensitizers and non-sensitizers.

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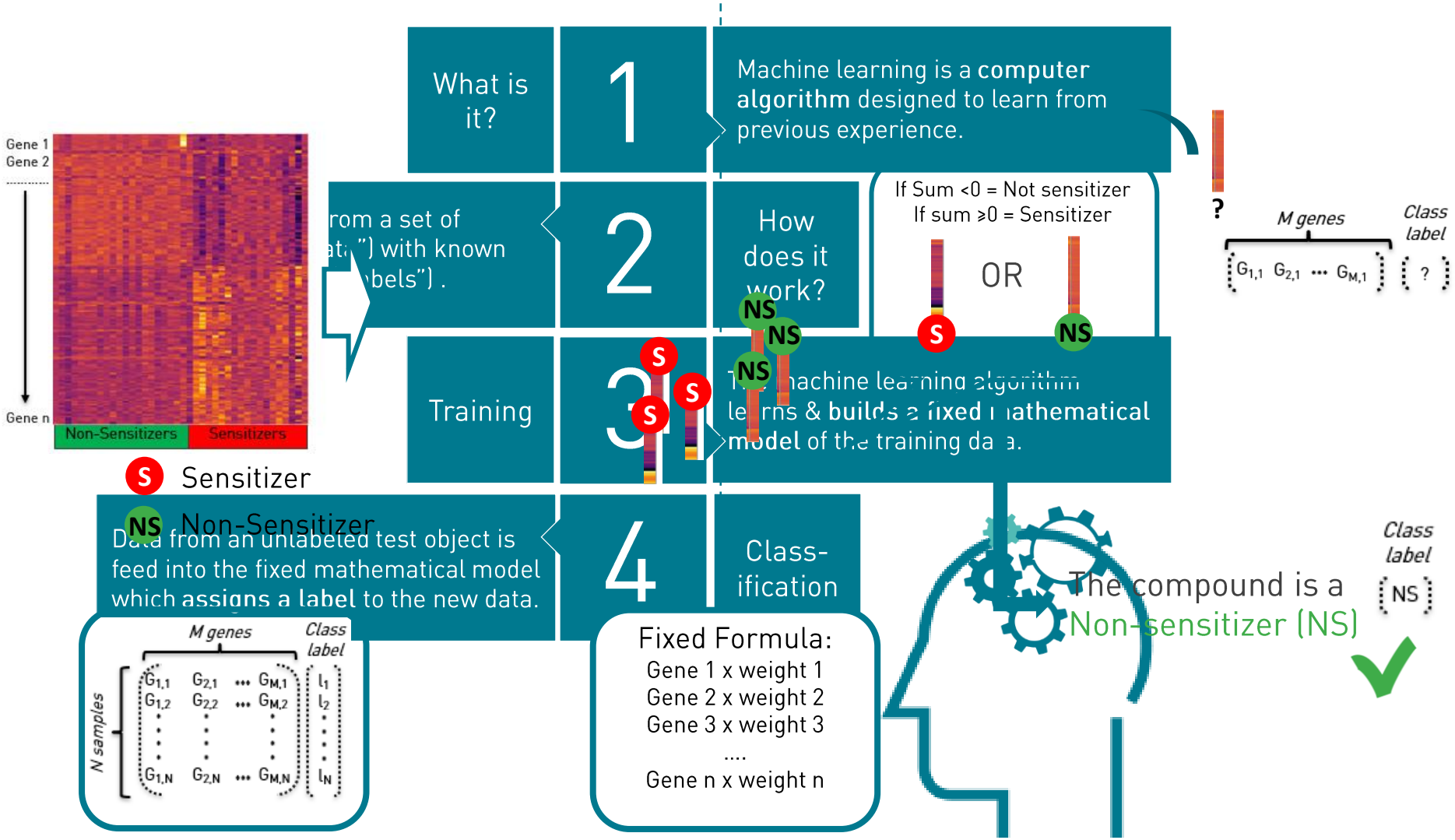
The GARD platform – Prediction model

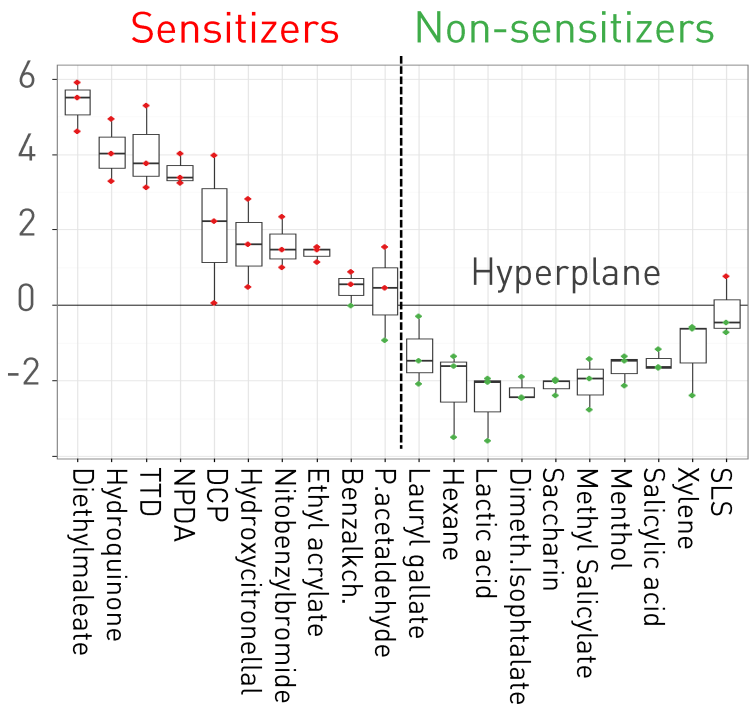


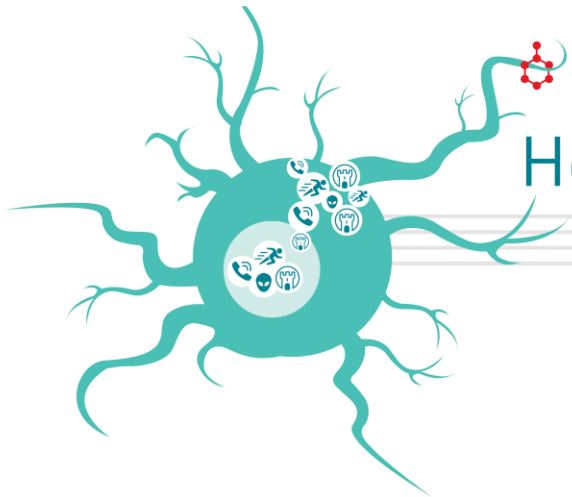
The GARD platform – Prediction model

Support Vector Machines (SVM)

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

? Classified as a Sensitizer

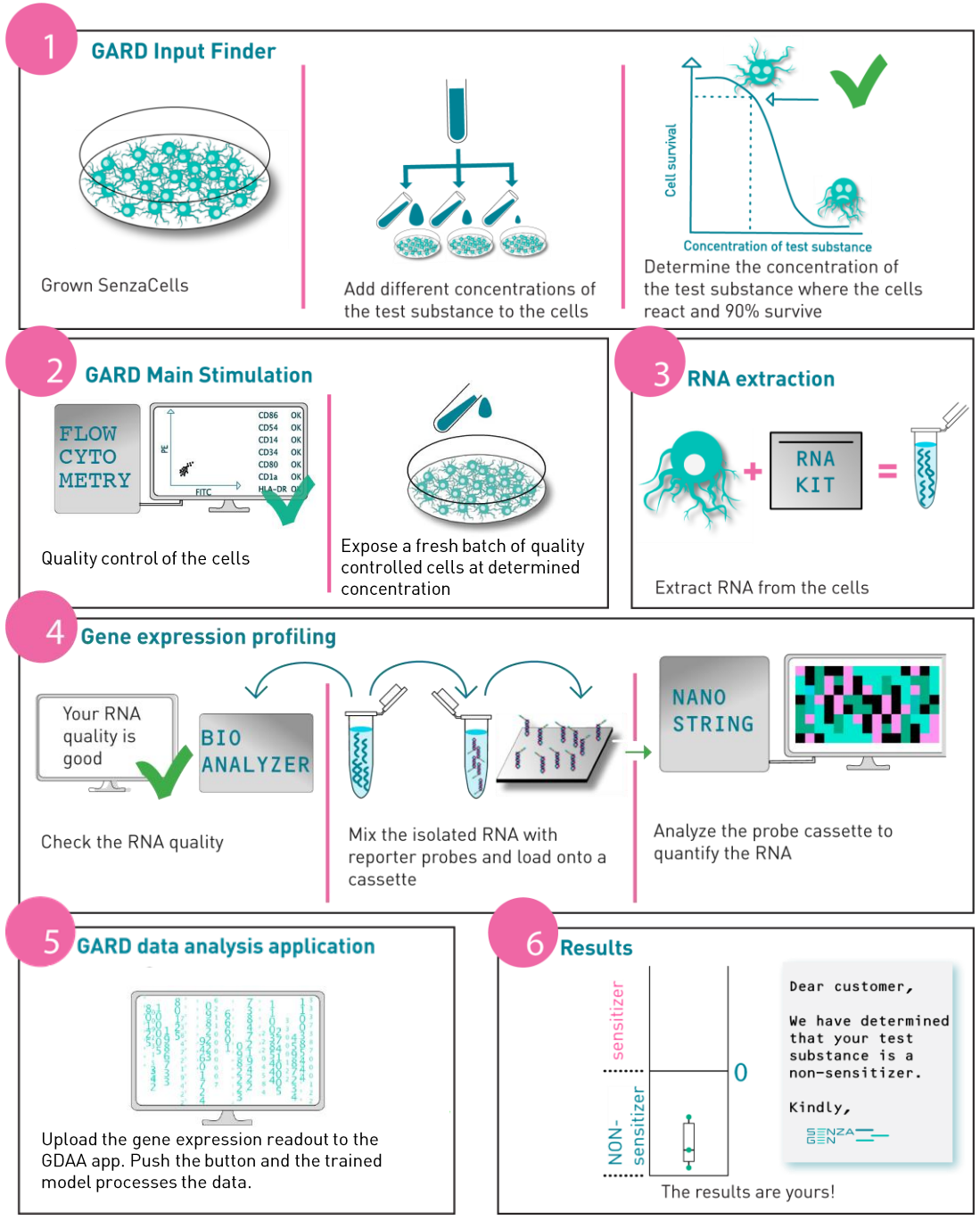




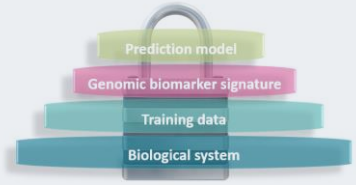
How to **GARD™** your product in six steps

How it works

GARD in 6 Steps

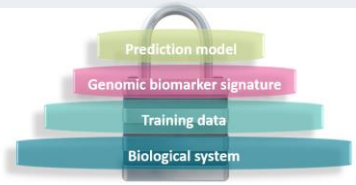


GARD: available assays



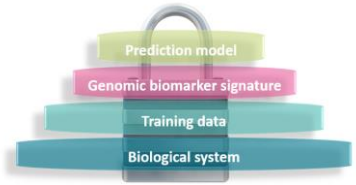
GARD™skin – 200 genes

Skin sensitization testing



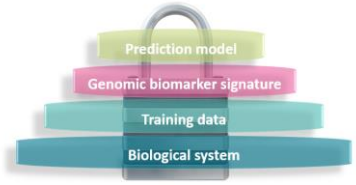
GARD™potency – 51 genes

Skin sensitization potency testing according to GHS/CLP



GARD™air – 28 genes

Respiratory sensitization testing



GARD™skin Medical Device – 200 genes

Skin sensitization testing of medical devices

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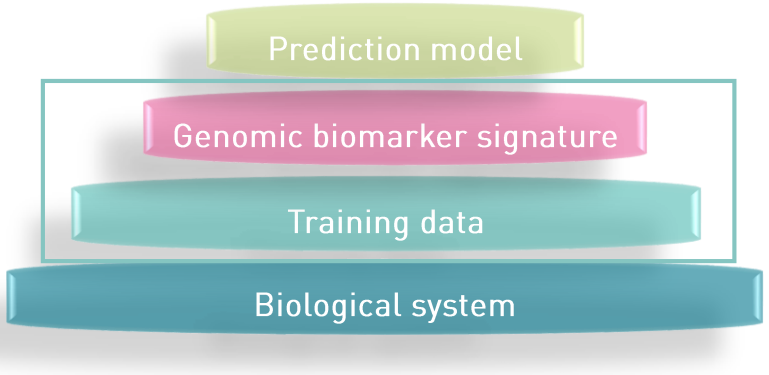
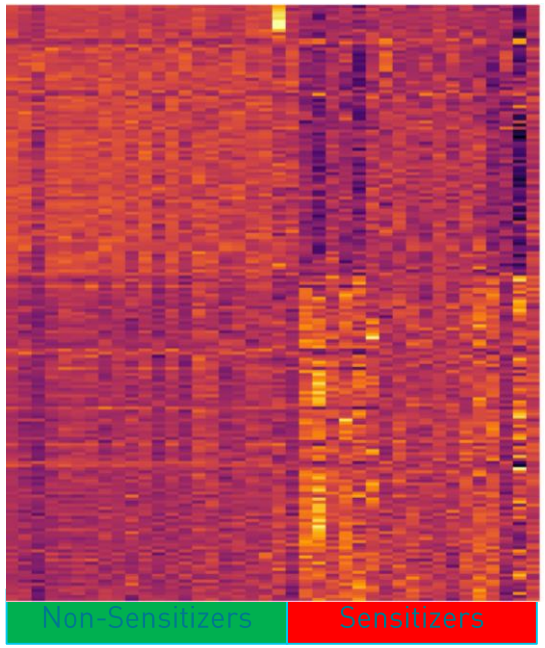
GARDskin: assay development

Training Data set

20 Skin sensitizers
20 Non-sensitizers

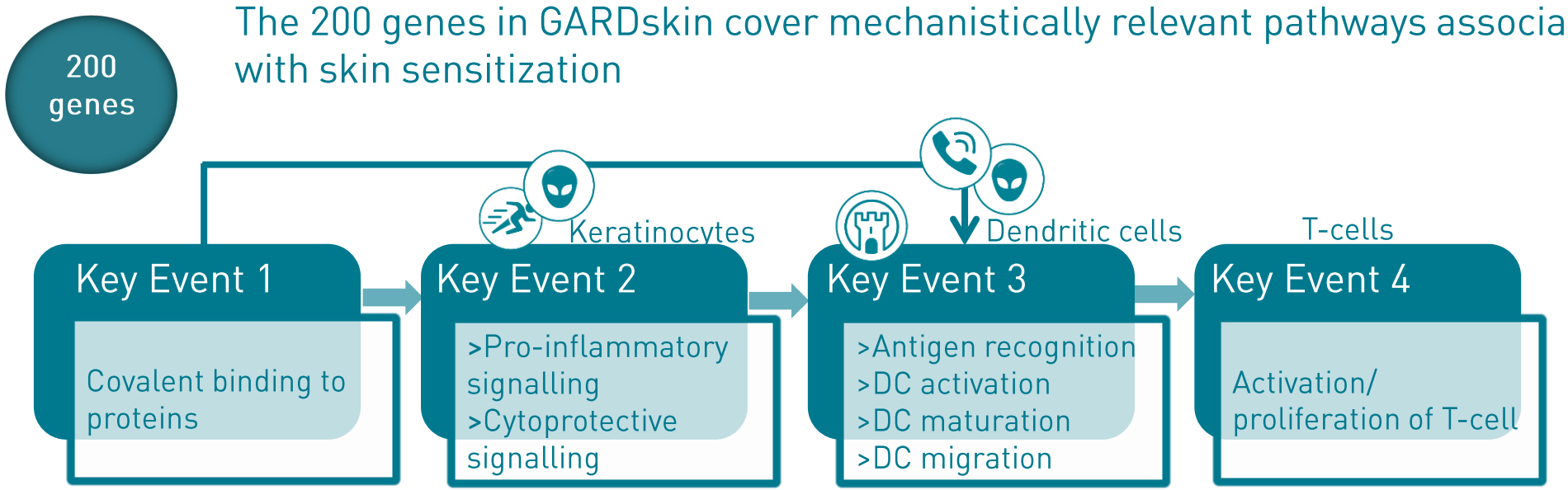


GARDskin prediction signature (GPS)



200 genes

GARDskin: assay development



GARDskin: assay development

Full transparency:

All assay development steps & the complete list of genes have been published.

Gene Symbol	Gene Title	Accession	ID	NanoString Probe ID
ABAT	4-aminobutyrate aminotransferase	18	7993126	NM_020686.5:268
ABHD5	abhydrolase domain containing 5	51099	8079153	NM_016006.4:910
ACER2	alkaline ceramidase 2	340485	8154563	NM_001010887.2:1306
ACLY	ATP citrate lyase	47	8015460	NM_001096.2:3990
ACTR10	actin-related protein 10 homolog (S. cerevisiae)	55860	7974587	NM_018477.2:1016
ADAM20	ADAM metallopeptidase domain 20	8748	7979927	NM_003814.4:1420
ALDH18A1	aldehyde dehydrogenase 18 fam., member A1	5832	7935230	NM_001017423.1:2617
ALDH1B1	aldehyde dehydrogenase 1 fam., member B1	219	8155327	NM_000692.3:1255
ANAPC1	anaphase promoting complex subunit 1	64682	8043349	NM_022662.3:7202
ANAPC5	anaphase promoting complex subunit 5	51433	7967149	NM_016237.4:1444
ANKRA2	ankyrin repeat, fam. A (RFXANK-like), 2	57763	8112596	NM_023039.4:741
ARFGAP3	ADP-ribosylation factor GTPase activating protein 3	26286	8076515	NM_001142293.1:2362
ARHGAP9	Rho GTPase activating protein 9	64333	7964436	BC006107.1:1808
ASB7	ankyrin repeat and SOCS box-containing 7	140460	7986433	NM_024708.3:1280
ATP6V0D1//ATP6V0D1	ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d1//ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d1	9114//9114	8002041	NM_004691.4:1101
ATP6V0E1	ATPase, H+ transporting, lysosomal 9 kDa, V0 subunit e1	8992	8110022	NM_003945.3:617
ATP6V1H	ATPase, H+ transporting, lysosomal 50/57 kDa, V1 subunit H	51606	8150797	NM_213620.2:1095
BCL7A	B-cell CLL/lymphoma 7A	605	7959354	NM_001024808.1:594
BIN2	bridging integrator 2	51411	7963289	NM_016293.2:855
BLMH	bleomycin hydrolase	642	8014008	NM_000386.3:2088
BXDC1//RPF2	brix domain containing 1//ribosome production factor 2 homolog (S. cerevisiae)	84154//84154	8062211	ENST00000424137.1:288
C11orf61	chromosome 11 open reading frame 61	79684	7952445	NM_024631.2:1622

BMC Genomics

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

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

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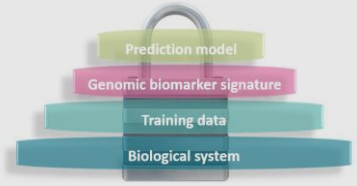
GARD: available assays

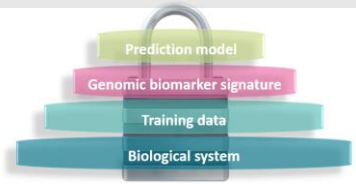
GARD™skin – 200 genes

Skin sensitization testing



GARD™potency – 51 genes

Skin sensitization potency testing according to GHS/CLP



GARD™air – 28 genes

Respiratory sensitization testing

GARD™skin Medical Device – 200 genes

Skin sensitization testing of medical devices

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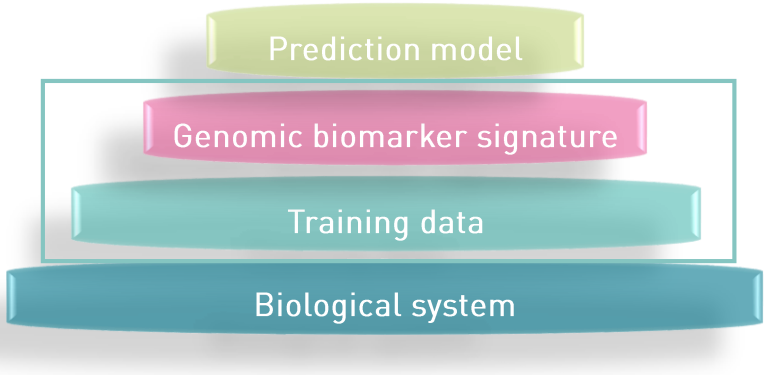
GARDpotency: assay development

Training Data set

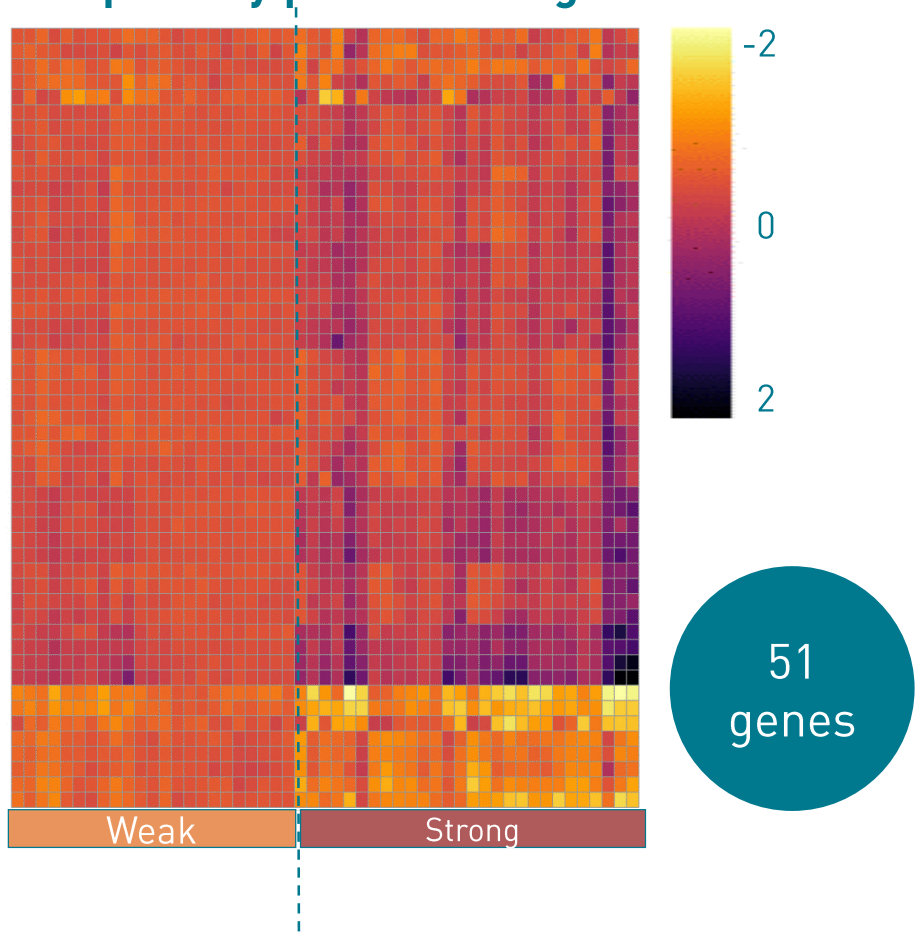
22 Class 1A (Strong Sensitizers)
29 Class 1B (weak-sensitizers)



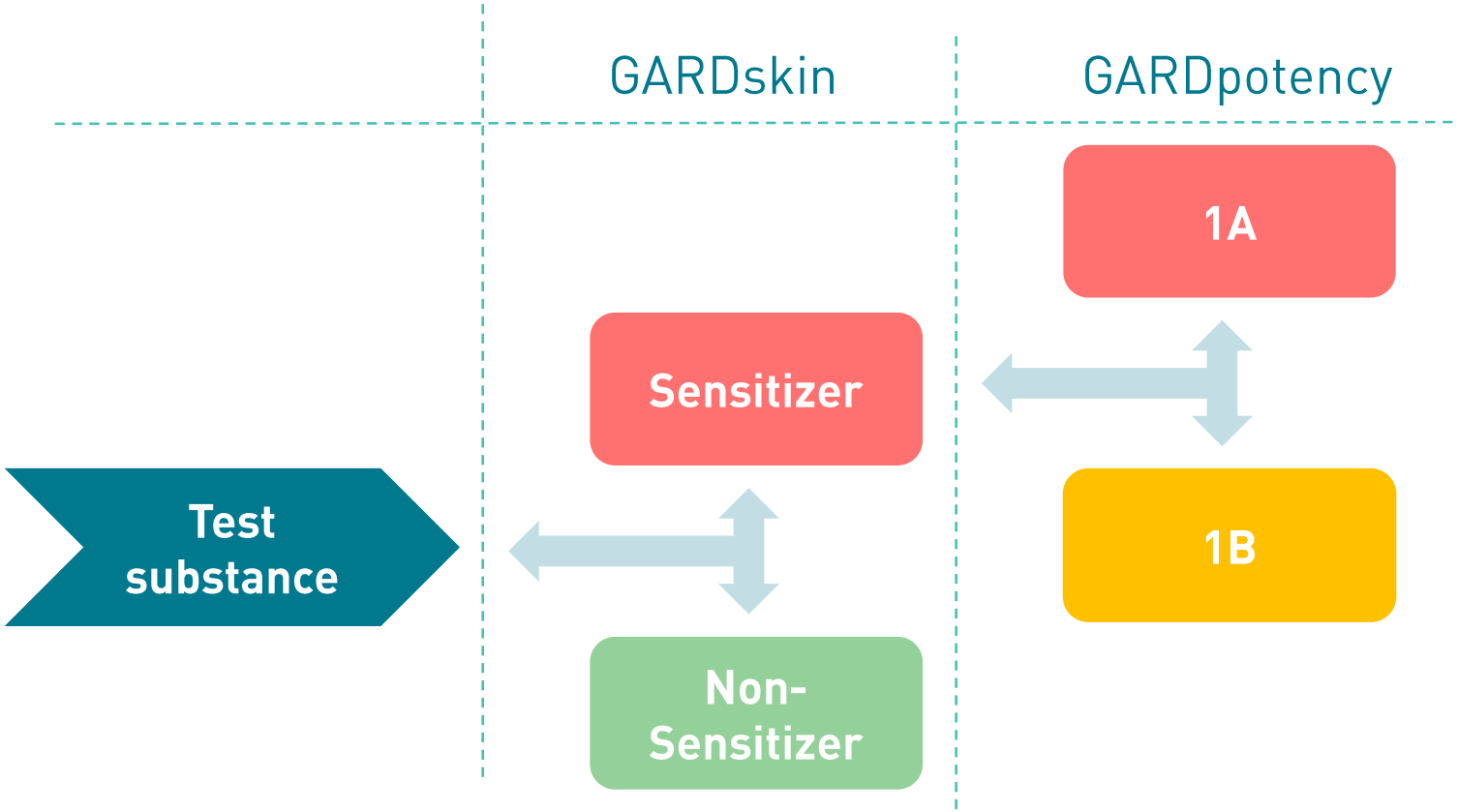
The GARD platform for potency assessment of skin sensitizing chemicals

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

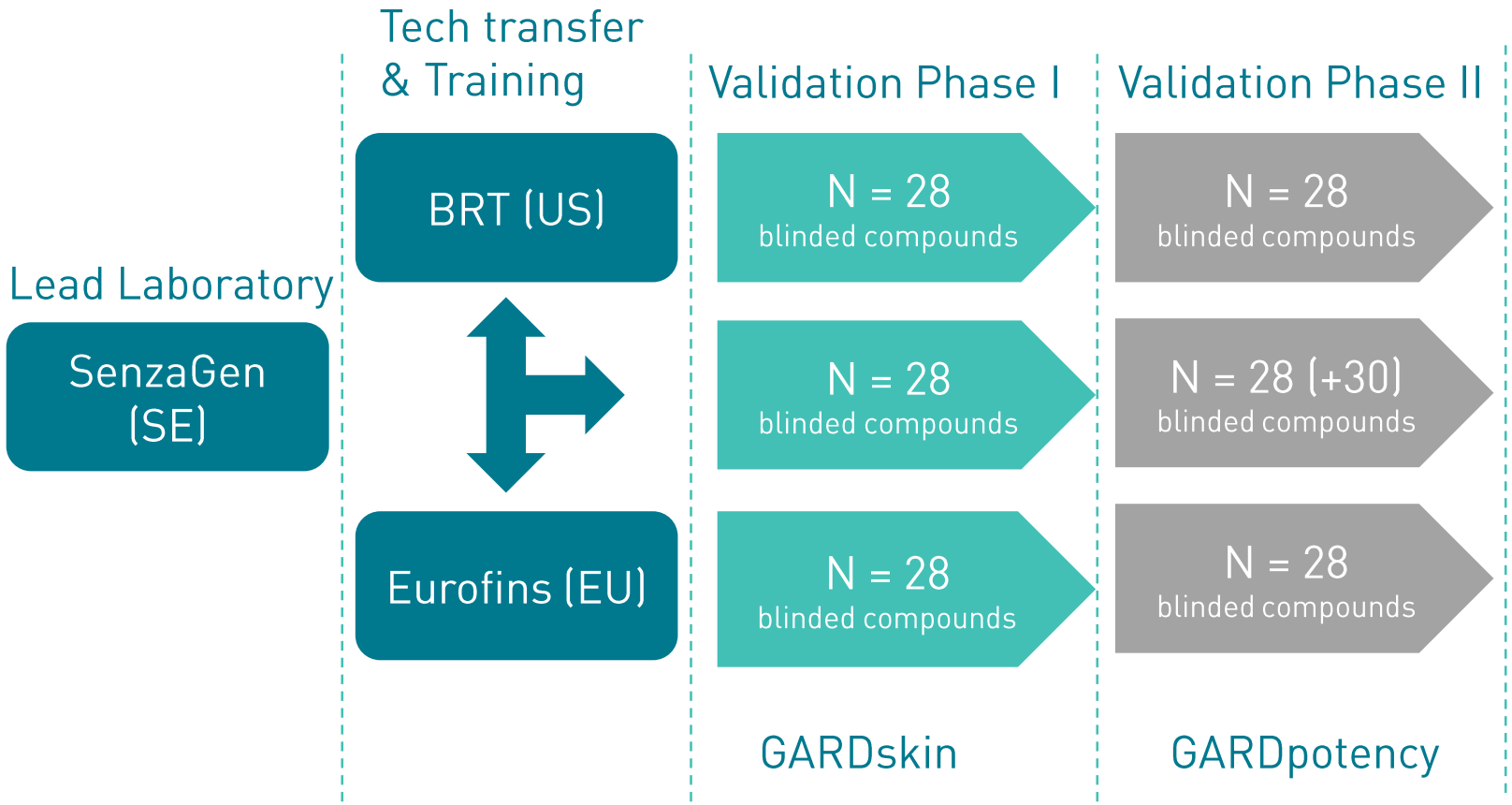
GARDpotency prediction signature



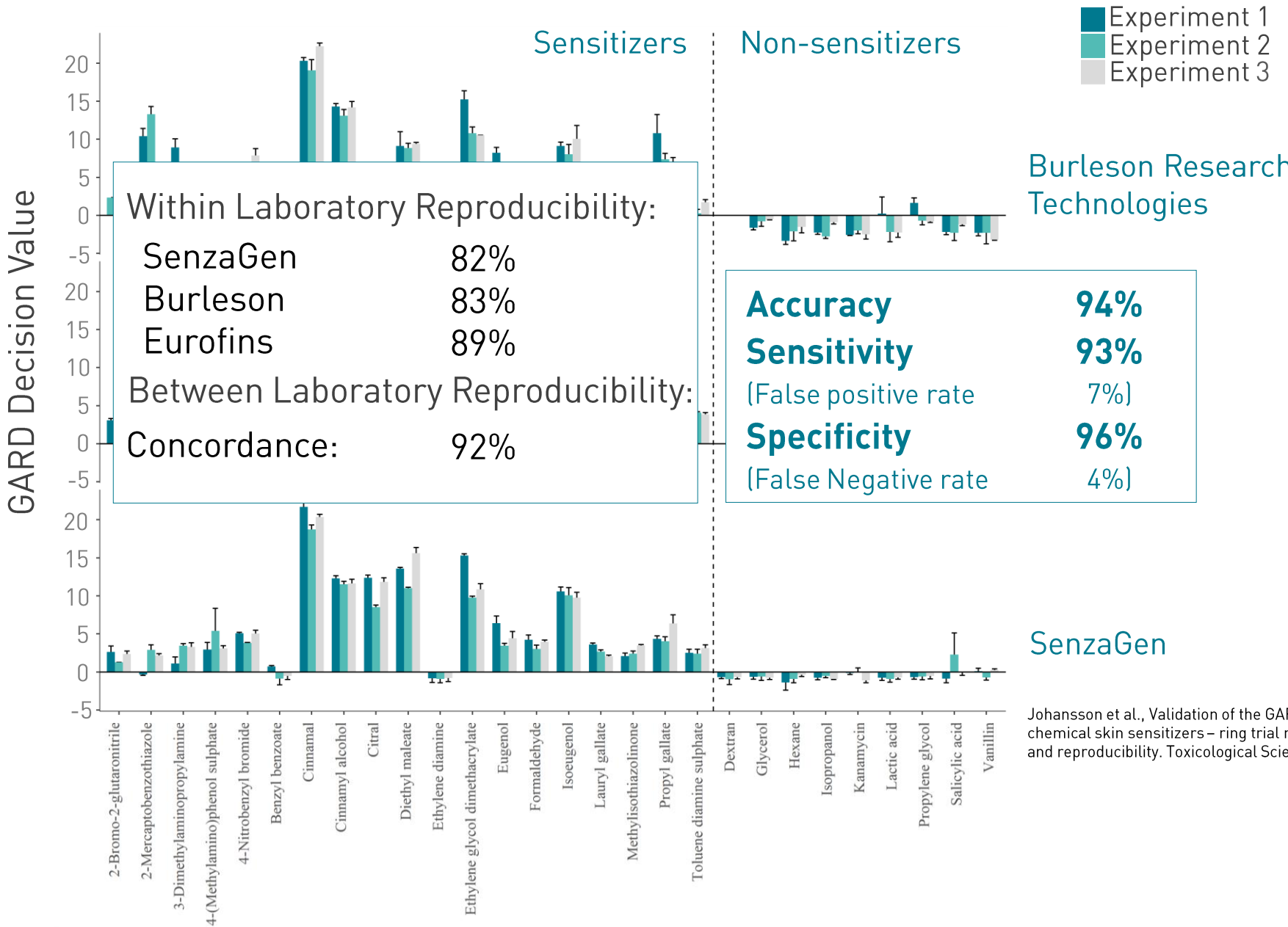
GARDpotency: subclassification of skin sensitizers according to GHS/CLP



Validation study: GARDskin & GARDpotency



Validation results: GARDskin



Within Laboratory Reproducibility:

SenzaGen	82%
Burleson	83%
Eurofins	89%

Between Laboratory Reproducibility:

Concordance:	92%

Burleson Research Technologies

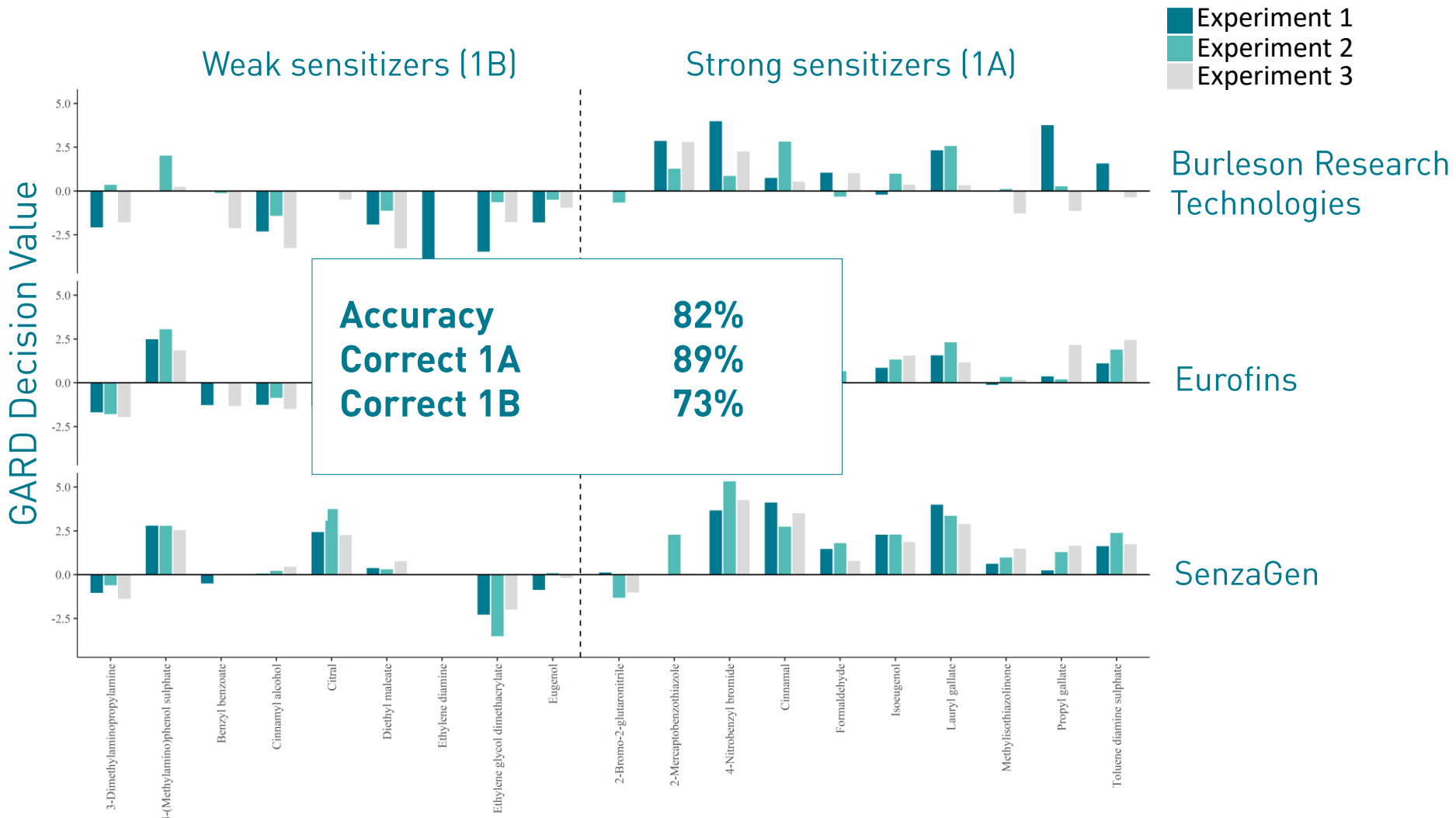
Accuracy	**94%**
Sensitivity	**93%**
(False positive rate	7%)
Specificity	**96%**
(False Negative rate	4%)

SenzaGen

Johansson et al., Validation of the GARD™skin assay for assessment of chemical skin sensitizers – ring trial results of predictive performance and reproducibility. Toxicological Sciences. May 17, 2019

SENZA GEN

Validation results: GARDpotency



GARDskin & GARDpotency: REACH registration

"The REACH Regulation allows the use of non-adopted in vitro methods in case they meet the EURL ECVAM criteria for entering pre-validation. For the GARD assay this criteria is met, as it is currently being validated."

"The current REACH information requirements require that three KEs are examined and GARD assay can be used to assess the KE 3."

Included in OECD TGP 4.106
Accepted by ECHA for REACH dossiers

ECHA
EUROPEAN CHEMICALS AGENCY

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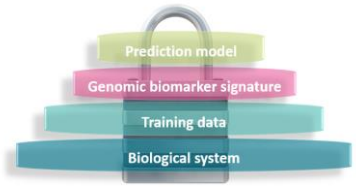
GARD: available assays

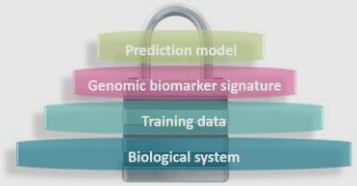
GARD™skin – 200 genes

Skin sensitization testing



GARD™potency – 51 genes

Skin sensitization potency testing according to GHS/CLP



GARD™air – 28 genes

Respiratory sensitization testing

GARD™skin Medical Device – 200 genes

Skin sensitization testing of medical devices

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GARDair: identification of chemical respiratory sensitizers

EU Commission – Flagship Product Health :

"GARDair – The first predictive in vitro assay for the identification of respiratory sensitizers. "

€2.4 M in funding

This project has recieved funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 756014.

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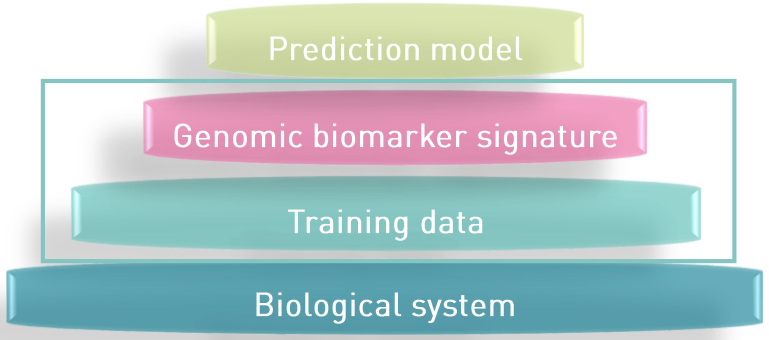
GARDair: assay development

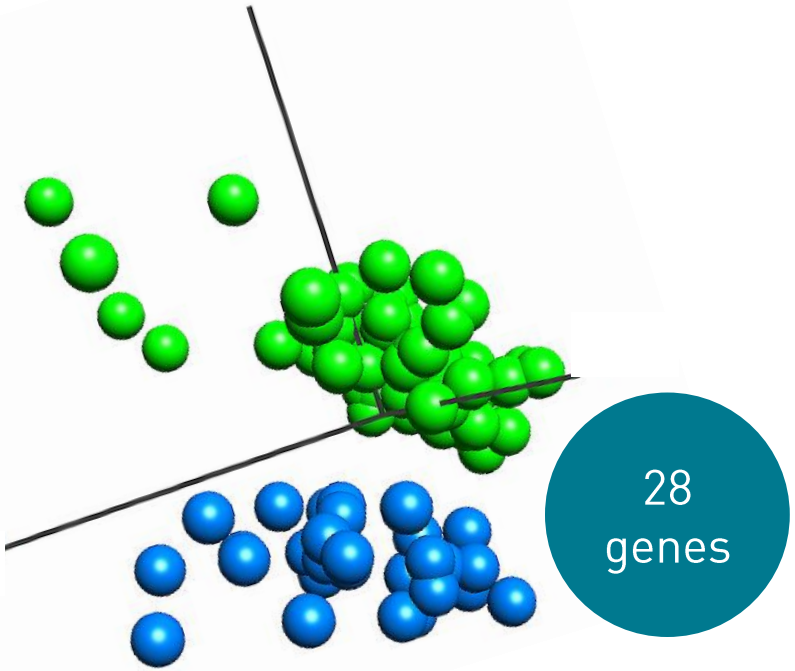
Training Data set

10 respiratory sensitizers
20 Non-respiratory sensitizers
(incl. skin sensitizers)



PLOS ONE | Forreryd A, Johansson H, Albrekt A-S, Borrebaeck CAK, Lindstedt M (2015)

GARDair prediction signature



Non-Respiratory sensitizers
Respiratory sensitizers

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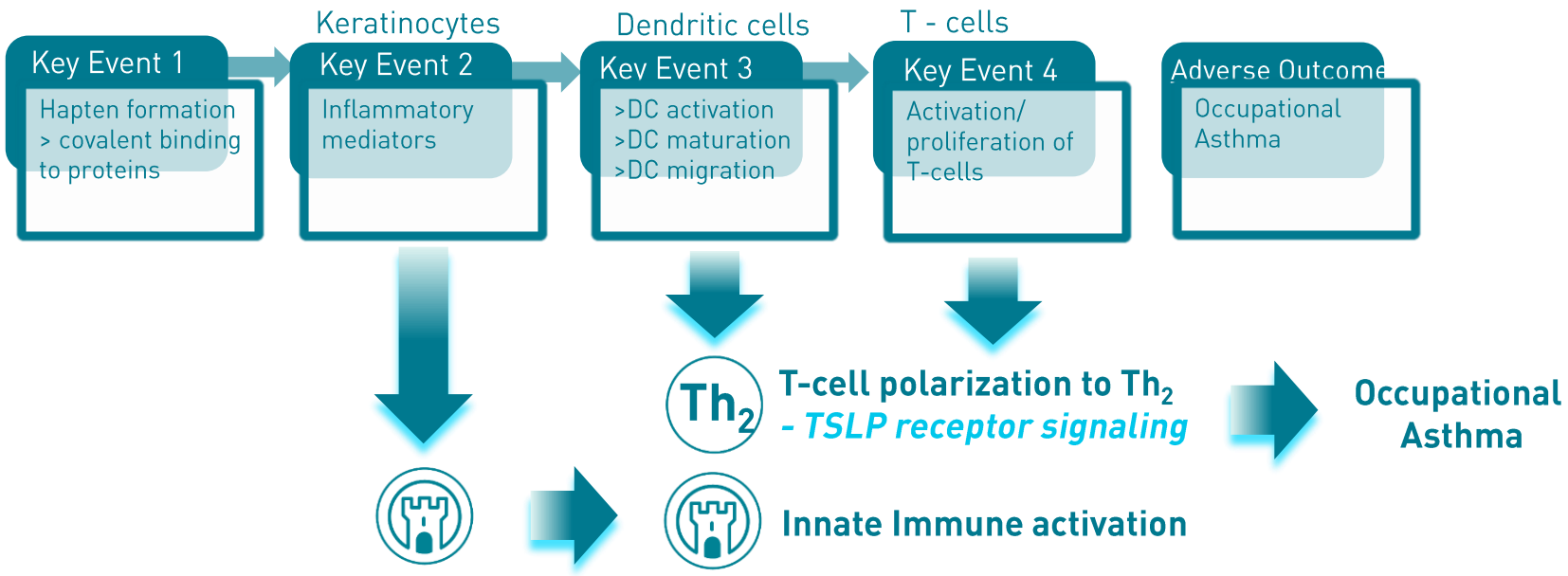
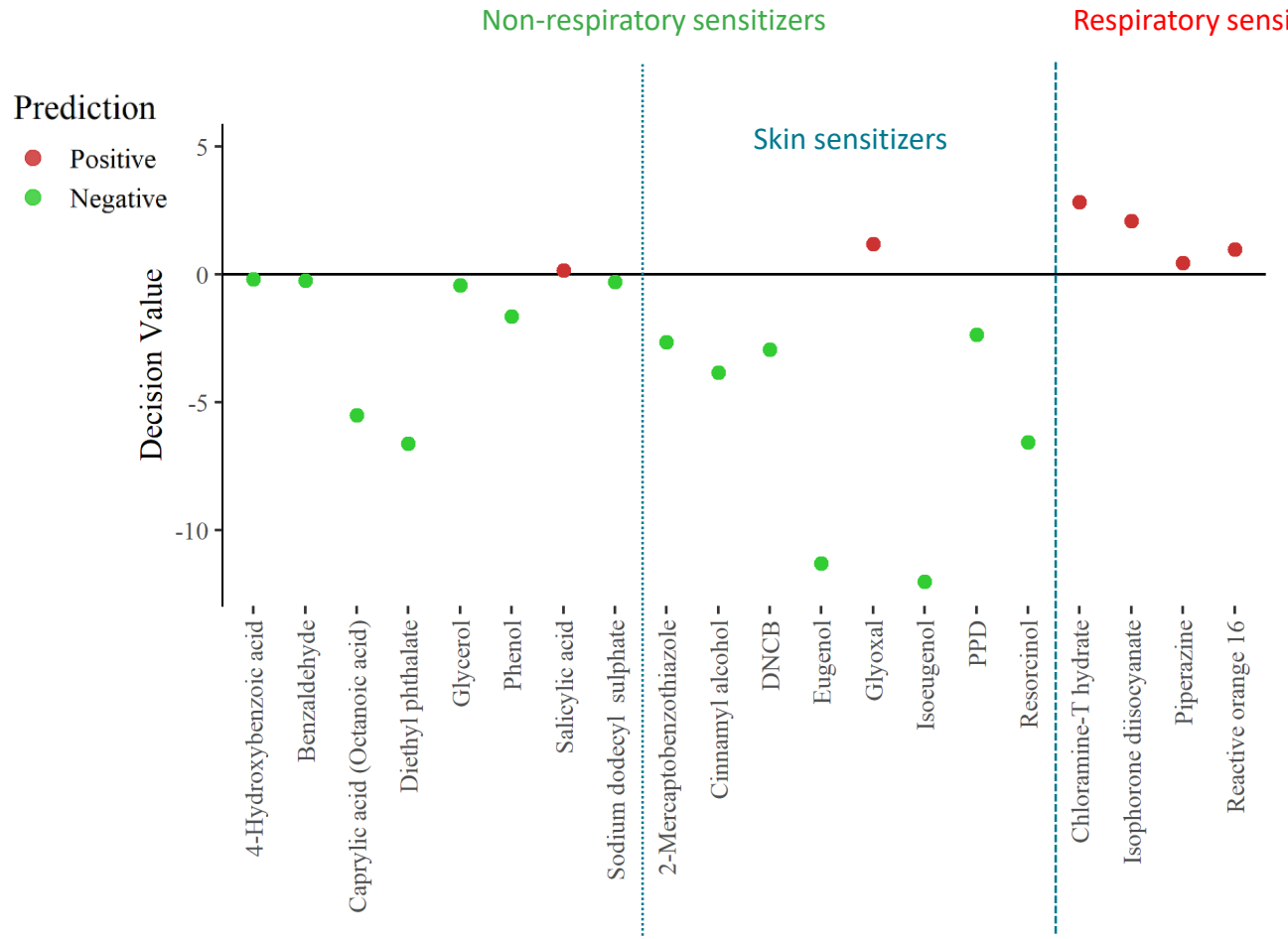
GARDair: coverage of mechanistically relevant pathways



GARDair: in house validation study results

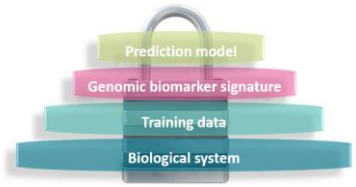


Accuracy	90%
Sensitivity	100%
Specificity	88%

GARD: available assays

GARD™skin – 200 genes

Skin sensitization testing



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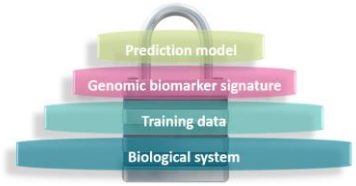
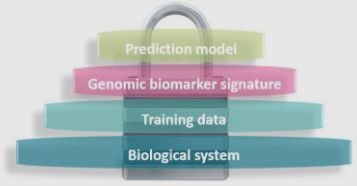
Skin sensitization potency testing according to GHS/CLP



GARD™air – 28 genes

Respiratory sensitization testing



GARD™skin Medical Device – 200 genes

Skin sensitization testing of medical devices

GARDskin Medical Device: assay development

Requirements

- Polar and non-polar extraction vehicles according to ISO 10993-12:2012
- Sensitive enough to detect low-level of skin sensitizers in extracts

Development

- Find oil that works with the assay
- Demonstrate sensitivity of the assay, by perform analysis on materials spiked with known skin sensitizers

GARDskin Medical Device: in house validation

Extraction vehicles

- Saline
- Super Refined Olive Oil
- Sesame oil, Ph Eur

Material

- Silicone and TPU spiked with five known skin sensitizers
- Tubes (Silicone, TPU and PVC)

Controls

- Negative control, vehicle control
- Positive control, vehicle spiked with P-phenylenediamine (PPD)

Extraction conditions

- 0.2 g/ml
- 37 ± 1°C for 72 ± 2h

GARDskin Medical Device: in house validation

Summary of the GARDskin Medical Device results from the materials used in this study compared with LLNA (as listed in the CE STTF database) and Human potency classification (HP) for the chemicals (Basketter et al. 2014)

Test material	Chemical	Sensitizing potential		GARD®skin Medical Device Prediction		
		LLNA	HP	Saline	Olive oil	Sesame oil
Silicone	None	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
	2-aminophenol	Strong	Cat 2	Sensitizer	Sensitizer	Sensitizer
	Cinnamic aldehyde	Moderate	Cat 2	Sensitizer	Sensitizer	Sensitizer
	Propyl gallate	Strong	Cat 2	Sensitizer	Sensitizer	Sensitizer
	Phenyl benzoate	Weak	Cat 3	Sensitizer	Sensitizer	Sensitizer
TPU	None	N/A	N/A	Non-sensitizer	Non-sensitizer	Not tested
	Propyl gallate	Strong	Cat 2	Sensitizer	Sensitizer	Not tested
	Phenyl benzoate	Weak	Cat 3	Sensitizer	Sensitizer	Not tested
Silicone tube	-	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
TPU tube	-	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
PVC tube	-	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
Vehicle control	-	Neg		Non-sensitizer	Non-sensitizer	Non-sensitizer
Positive control	p-Phenylenediamine	Pos		Sensitizer	Sensitizer	Sensitizer

Conclusion: All items were predicted correctly

The spiked materials were produced by Research Institute of Sweden (RISE)
The tubes are supplied by Medizintechnik Promedt

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Why use GARD™?

Features and benefits

Compliance

Regulatory and quality standards

OECD

- GARDskin and GARDpotency included in OECD Test Guideline Program (TGP no. 4.106).

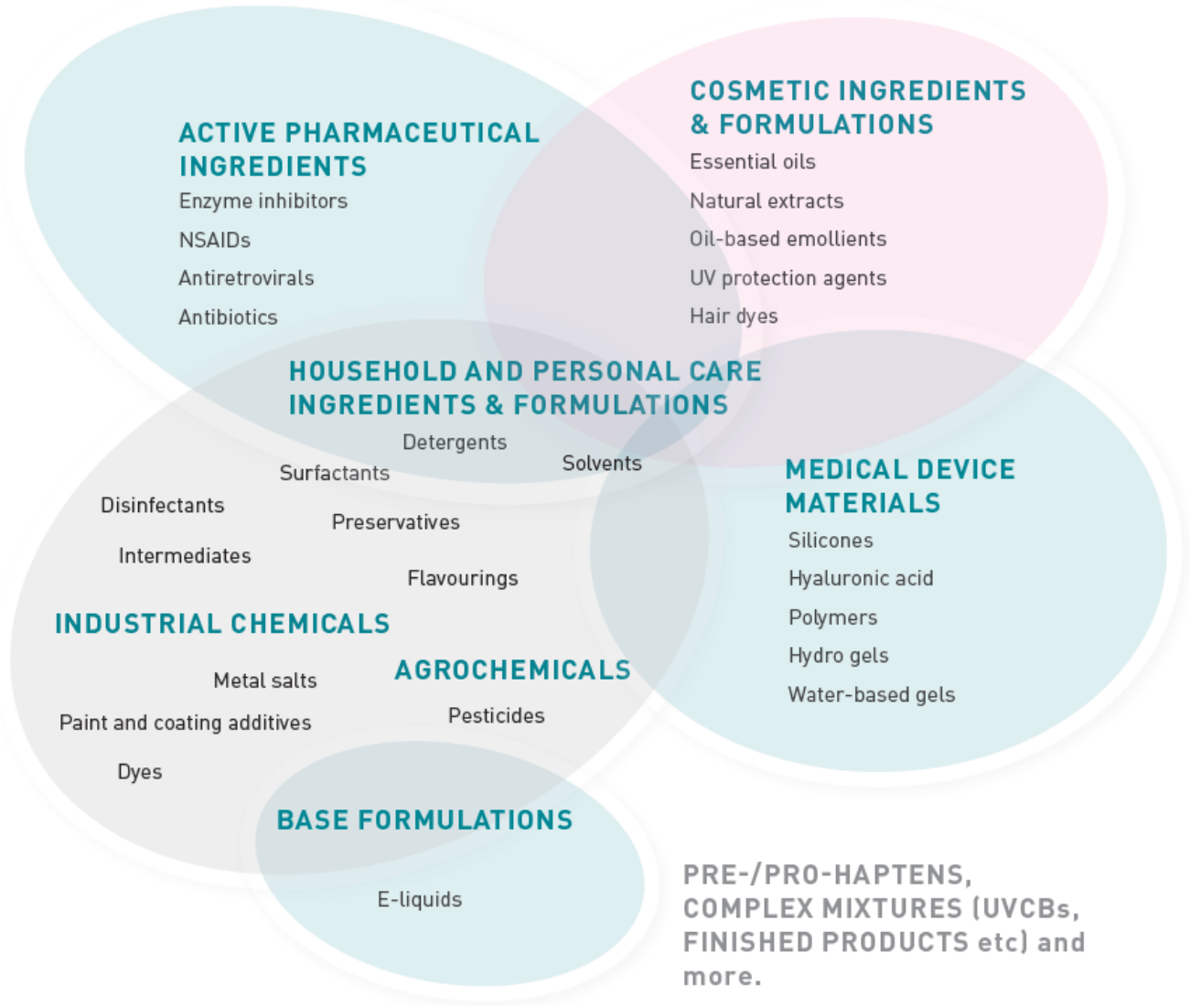
REACH and CLP

- Accepted by ECHA for REACH dossiers.
- REACH registration of chemicals
- CLP 1A &1B potency classification

GLP

- GLP audit and approval expected in spring 2020

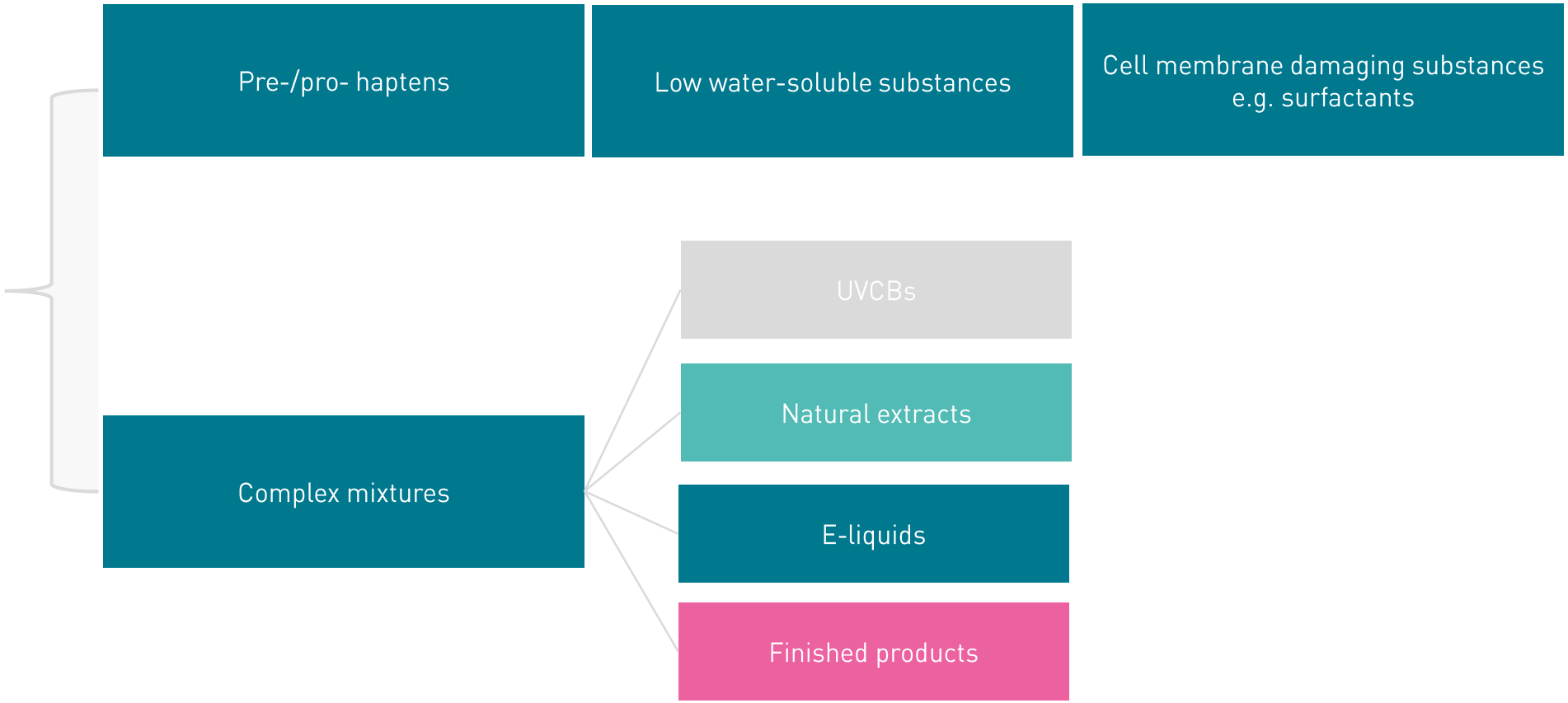
Broad applicability



Over 400 items successfully tested on GARD

The list contains examples of items that have been tested on the GARD platform as part of internal validation studies or customer projects.

Expertise in “difficult-to-test samples”



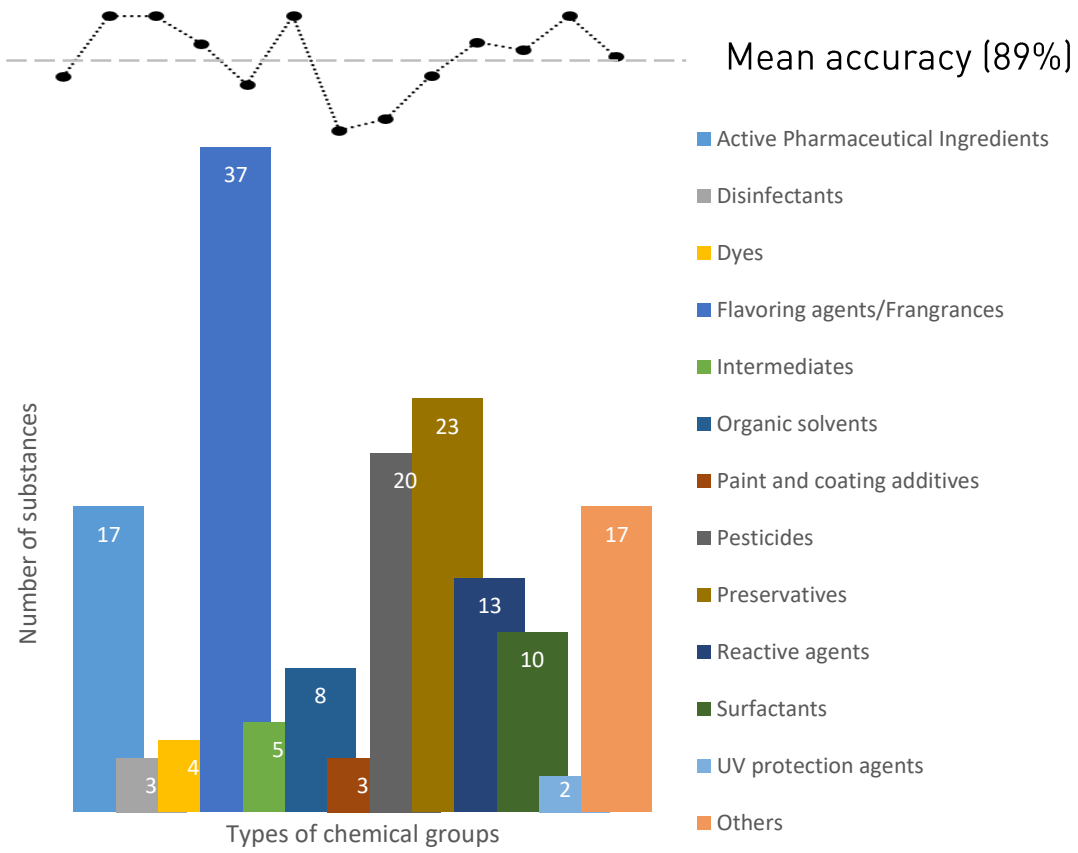
GARDskin: suitable for “challenging substances”

GARDskin is applicable for a wide range of chemical ingredients used in

- Industrial chemicals
- Agrochemicals
- Cosmetics
- Pharmaceuticals
- Household & personal care products

Including “challenging substances” such as:

- Pre-/Pro-haptens
- Low water-soluble substances
- Substances with cell membrane damaging properties, e.g. surfactants.
- UVCBs with known average molecular weight



* Data of 162 substances all come from internal validation studies, excluding customer projects

GARDskin: pre-/pro-haptens, low water-soluble substances

Compound	Pre/pro hapten	High logP	DPRA (TG442C)	ARE-NRF2 (TG 442D)	h-CLAT (TG442E)	GARD (TGP 4.106)
2-Aminophenol	YES	-	S	S	S	S[1]
2-nitro-1,4-Phenylendiamine	YES	-	S	S	S	S[1]
Aniline	YES	-	NS	NS	S	S[1]
Cinnamic alcohol	YES	-	S	S	S	S[1]
Ethylene diamine	YES	-	NS	S	S	NS[1]
Eugenol	YES	-	S	NS	S	S[1]
Geraniol	YES	-	NS	S	S	S[1]
Hydroquinone	YES	-	S	S	S	S[2]
Isoeugenol	YES	-	S	S	NS	S[1]
Linalool	YES	-	NS	NS	S	S[1]
p-Phenylenediamine	YES	-	S	S	S	S[1]
Propyl gallate	YES	-	S	S	S	S[1]
Resorcinol	YES	-	NS	NS	S	S[1]
Farnesol	YES	-	NS	S	S	S[1]
Abietic acid	YES	YES (6.5)	S	S	NS	S[1]
Chlorpromazine	YES	YES (5.4)	NA	NS	S	S[1]
Lauryl gallate	YES	YES (6.2)	S	S	S	S[3]
Amylcinnamyl alcohol	YES	YES (4.4)	S	NS	NS	S[1]
Limonene	YES	YES (4.6)	NS	NS	S	S[1]
Benzoyl peroxide	-	YES (3.5)	NS	NS	S	S[3]
Hexylcinnamic aldehyde	-	YES (4.8)	S	NS	NS	S[1]
Isopropyl myristate	-	YES (8.5)	NS	NS	S	NS[4]
propyl paraben*[1]	-	YES (3.4)	NS	S	S	S[1]
Tocopherol	-	YES (6.9)	NS	S	NS	S[1]
Accuracy			**61%**	**58%**	**71%**	**92%**

[1] Johansson et al. 2017, [2] Forreryd et al. 2016, [3] Zeller et al. 2017, [4] Johansson et al. 2019
*Basketter, Human potency Class 5

Challenge

- Pre-/pro-haptens need to be activated
- Pro-haptens more difficult to identify using in vitro test systems
- Low solubility in aqueous media

Our solution

- Metabolic activation for pro-haptens included in the test system
- Broad solvent alternatives
- Sensitive test system – only requires a small amount of sample that dissolves

Performance

- Average accuracy from published studies: 92%

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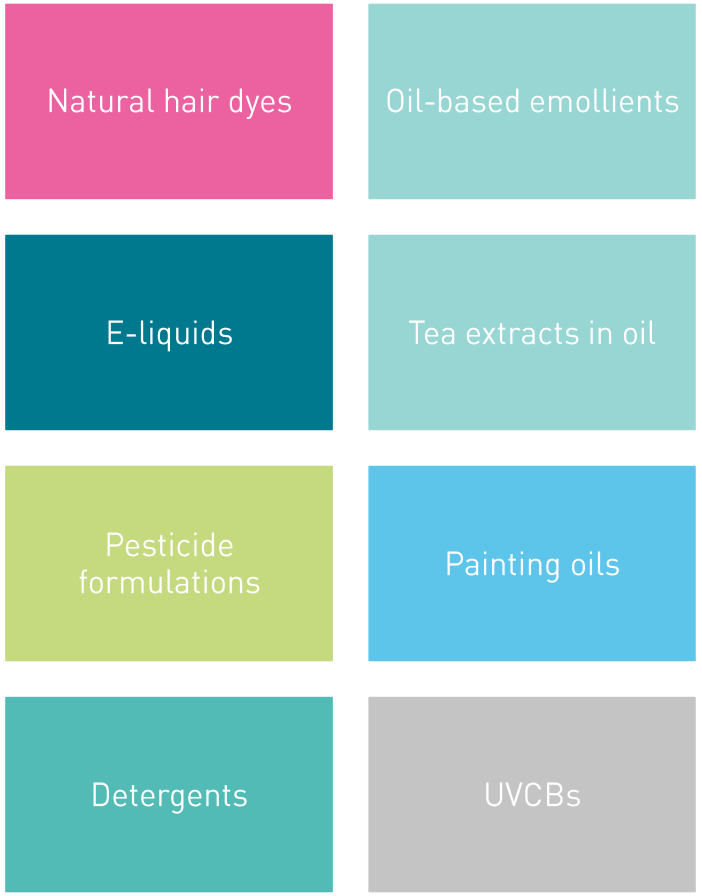
GARDskin: how about complex mixtures?



Examples of successfully tested complex mixtures

Challenge - Why difficult to test?

- Complexity and uncertainty of the compositions
- Solubility issues

Our solution

- Using average molecular weight to estimate the sample concentration
- Additional solubility tests can be performed to select suitable solvents

Complex mixtures successfully tested on GARDskin:

- Natural extracts
- Fragrances and flavouring formulations
- Agrochemical formulations
- Finished cosmetic/household products
- UVCBs

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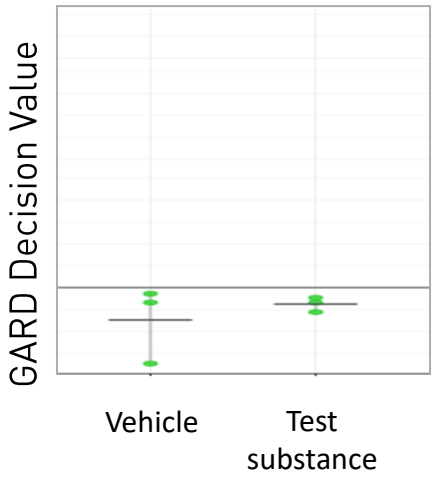
Available solvents for GARD

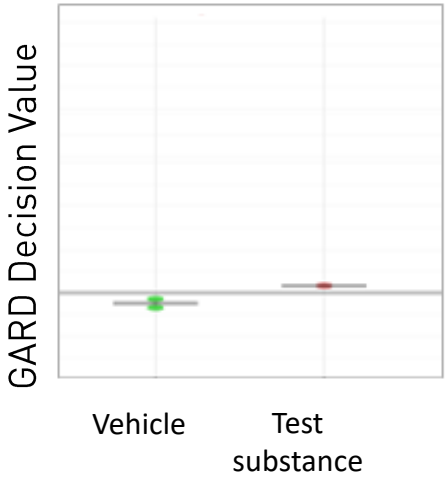
Acetone | DMF | DMSO | Isopropanol | Ethanol | Glycerol | Olive oil | Sesame oil

Vehicle: DMSO



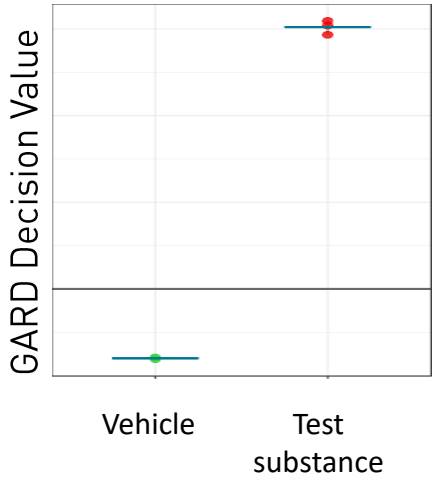
Not soluble

Vehicle: Water



Not soluble

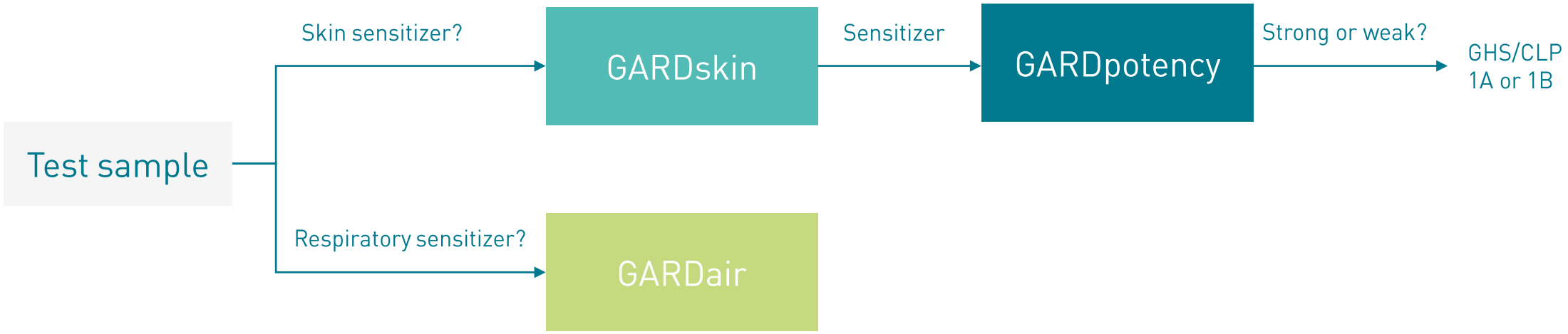
Vehicle: DMF/Glycerol



Soluble

Versatility

One sample several readouts



Efficiency

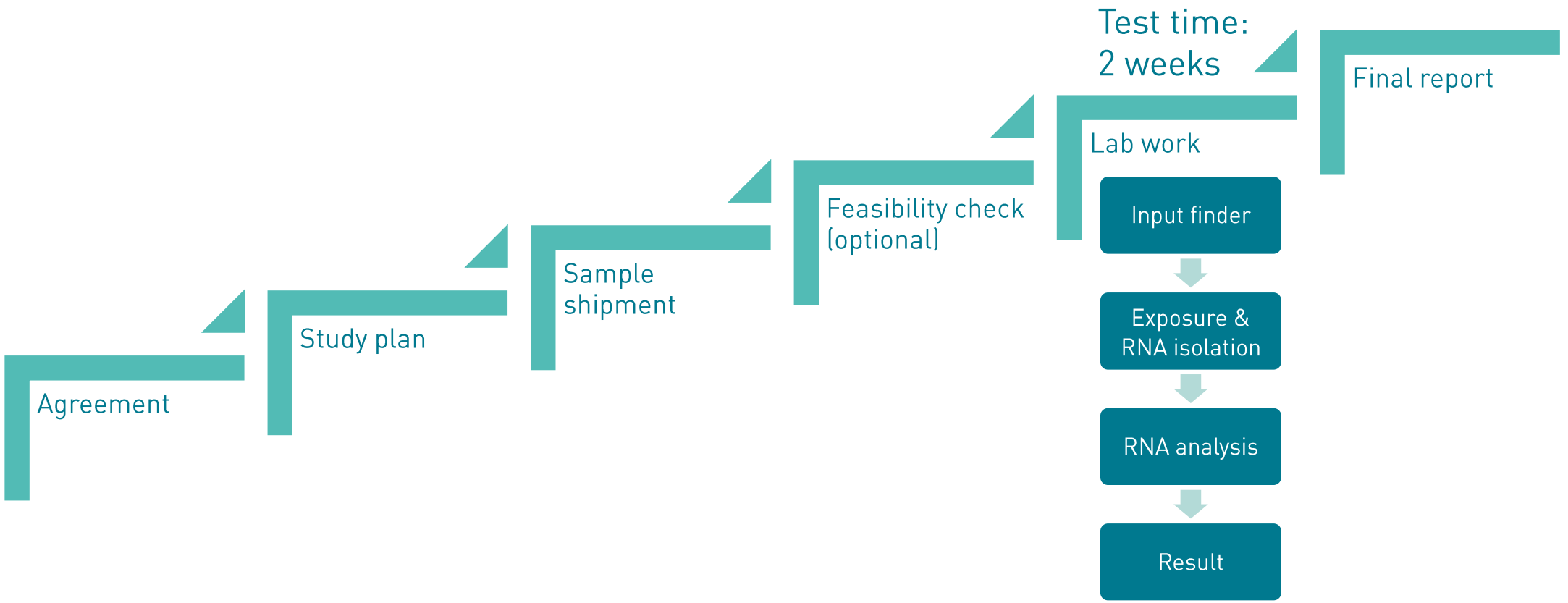
Time and cost

- Quick and reliable results.
- Short test time: 2 weeks.
- Less expensive than animal testing.
- “Pre-test” available for difficult-to-test samples

Sample requirements

- Only 0.5 g (solids) or 1 ml (liquids).
- Potential to be adapted to significantly lower amounts if needed.

Study timeline overview



Where to get my sample tested?

SenzaGen and its partners over the world

Sweden
SenzaGen AB

License Labs

Germany
Eurofins BPT

US
Burleson Research Technologies
MB Research Laboratories

Distributors - Europe

France
Eurosafe
GenEvolutioN
PKDerm

Italy
VitroScreen

The Netherlands
Charles River Laboratories

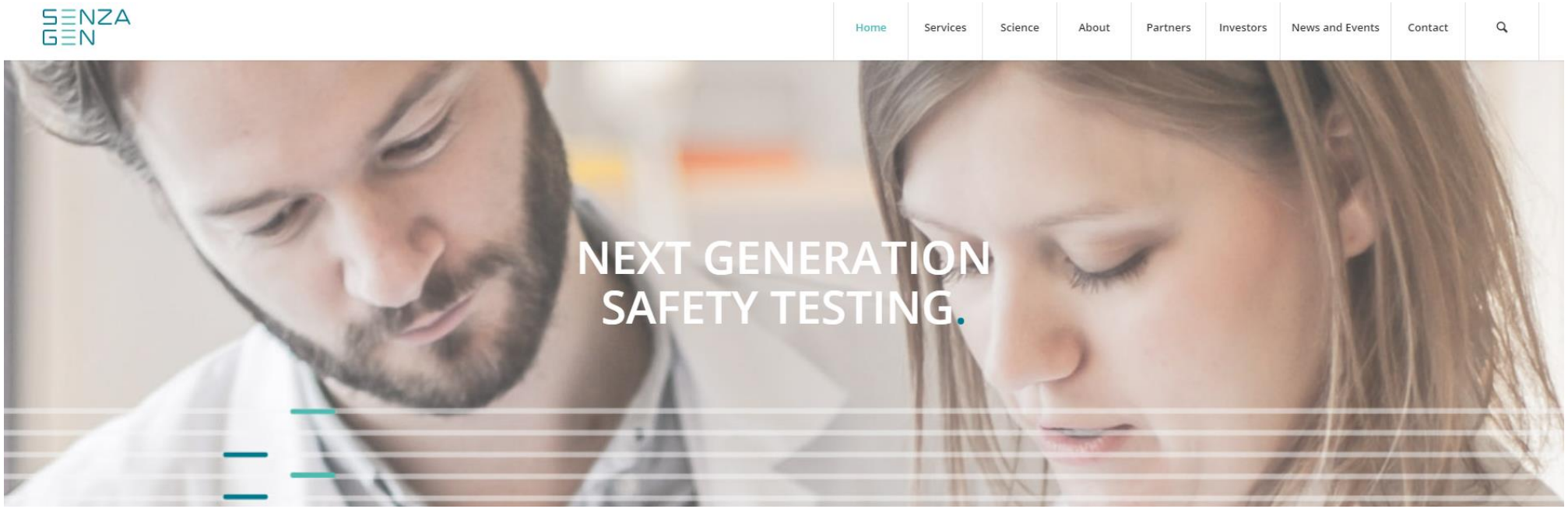
UK
XCellR8

Distributors - Asia

China
Guangzhou CHN-ALT Biotech Co., Ltd

South Korea
Woojung BSC

Contact us



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