GARDair – A novel assay for assessment of chemical respiratory sensitizers based on a genomic platform

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INTRODUCTION

Exposure to chemicals may induce allergic hypersensitivity reactions in skin or respiratory tract. To minimize exposure, chemicals are routinely screened for their sensitizing potential. Proactive identification has historically been performed using animal models, but the use of animals for safety assessment of cosmetics was recently banned within EU. Today, similar trends are spreading both globally and across industry and market segments. Methods for specific identification of respiratory sensitizers are greatly underdeveloped, with no validated, or even widely used assay readily available. Thus, there is an urgent need for development of non-animal-based methods for hazard classification of respiratory sensitizing chemicals.

GARD® – Genomic Allergen Rapid Detection – is a state of the art technology platform for assessment of chemical sensitizers (Figure 1). It is based on a dendritic cell (DC)-like cell line, thus mimicking the cell type involved in the initiation of the response leading to sensitization. Following test chemical exposure, induced transcriptional changes are measured to study the activation state of the cells. These changes are associated with the immunological decision-making role of DCs *in vivo* and constitutes of e.g. up-regulation of co-stimulatory molecules, induction of cellular and oxidative stress pathways and an altered phenotype associated with recognition of xenobiotic matter. By using state-of-the-art gene expression technologies, high informational content data is generated, that allows the user to get a holistic view of the cellular response induced by the test substance.

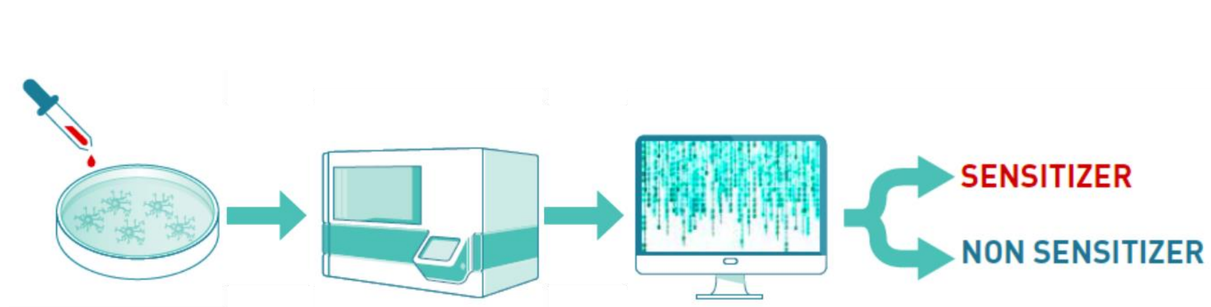

Figure 1. GARD – Genomic Allergen Rapid Detection. Chemically-induced changes in transcriptional levels of cells exposed to test chemicals are compared to predictive genomic biomarker signatures using supervised machine learning. Unknown samples are classified as either skin sensitizers, respiratory sensitizers or non-sensitizers based on output from the machine learning algorithm.

GARDair – SCIENTIFIC ORIGIN

GARDair is an application of the GARD platform®, designed for the specific assessment of respiratory chemicals. The assay was established by genome-wide gene expression analysis of DC-like cells exposed to a reference panel of chemicals, including respiratory sensitizers, skin sensitizers and non-sensitizers. Using a data-driven approach, comprising state-of-the-art bioinformatics and machine-learning assisted feature selection, a predictive genomic biomarker signature consisting of 389 transcripts, collectively referred to as the GARD Respiratory Prediction Signature (GRPS), was identified. A subsequent proof-of-concept study demonstrated the predictive capabilities of the identified biomarkers by the classification of an external test set, using a Support Vector Machine (SVM)-based classification algorithm (Figure 2).

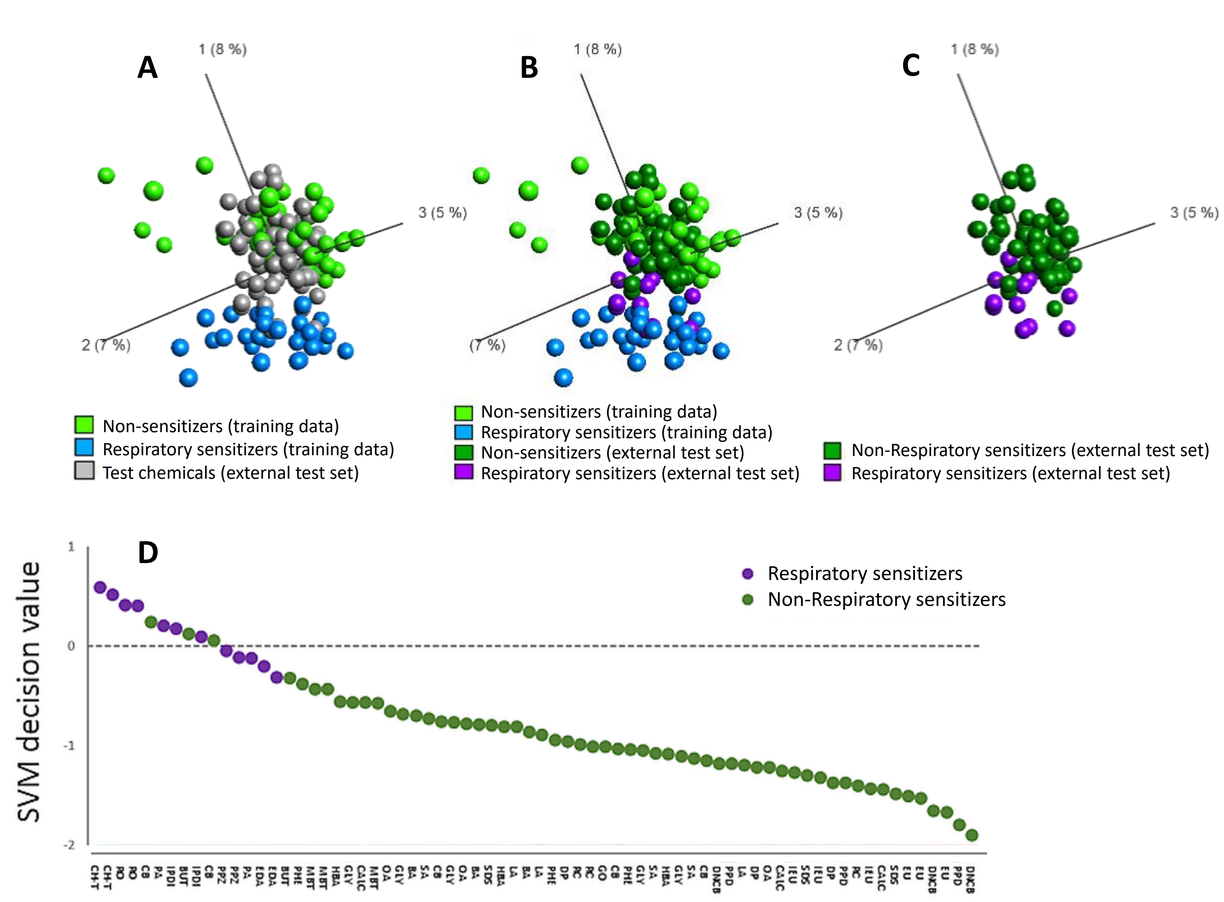

Figure 2. Visualization of the discriminatory capabilities of the respiratory sensitizer-specific biomarker prediction signature and subsequent classification of an external test set using principal component analysis (PCA). (**A**) The panel of reference chemicals used to identify the GRPS (training dataset) was used to generate the PCA space, using the 389 genes as variable input. The test set was plotted into the PCA space, without contributing to the principal components. (**B**) Test chemicals included in the test set are colored according to sensitizing properties. (**C**) The training dataset is removed to facilitate interpretation. (**D**) An SVM model was applied to predict samples in the test dataset. SVM mean decision values are plotted for each compound. The cutoff used for classification as respiratory sensitizers (SVM decision value > 0) is illustrated with a dashed line.

TECHNOLOGY PLATFORM TRANSFER

The validity of initial scientific results has recently been confirmed, and further optimization and industrial implementation is accelerated by funding from the EU Framework Programme for Research and Innovation Horizon 2020. The GRPS was optimized to allow both improved discrimination between respiratory sensitizers and non-sensitizers, as well as convenient technology platform transfer to a resource-effective, easy-to-use and fast gene expression analysis system. This was realized by the successful transfer of the assay to the Nanostring nCounter System.

Following model and assay optimization and the formalization and review of assay procedures in a Standard Operating Procedure (SOP), the finalized model was challenged with two external test sets, consisting of chemicals with well characterized respiratory sensitization properties (Table 1).

Table 1. GARDair classification results.

Chemical name	True group	GARDair Prediction Test set 1	GARDair Prediction Test set 2	Included in Training Set
2-Mercaptobenzothiazole	NRS	NRS	-	No
4-Hydroxybenzoic acid	NRS	NRS	-	No
Benzaldehyde	NRS	NRS	-	No
Octanoic acid	NRS	NRS	-	No
Chloramine-T hydrate	RS	RS	RS	No
Cinnamyl alcohol	NRS	NRS	-	No
Diethyl phthalate	NRS	NRS	-	No
DNCB	NRS	NRS	NRS	No
Eugenol	NRS	NRS	-	No
Glycerol	NRS	NRS	-	No
Glyoxal	NRS	RS	-	No
Isoeugenol	NRS	NRS	-	No
Isophorone diisocyanate	RS	RS	-	No
Phenol	NRS	NRS	-	No
Piperazine	RS	RS	RS	No
PPD	NRS	NRS	NRS	No
Reactive orange 16	RS	RS	RS	No
Resorcinol	NRS	NRS	-	No
Salicylic acid	NRS	RS	-	No
SDS	NRS	NRS	-	No
Chlorobenzene	NRS	-	NRS	Yes
DMSO	NRS	-	NRS	Yes
Maleic anhydride	RS	-	RS	Yes
Phenyl isocyanate (MDI)	RS	-	RS	Yes
Phthalic anhydride	RS	-	RS	Yes
Toluene diisocyanate	RS	-	NRS	Yes
Trimelitic anhydride	RS	-	RS	Yes

RS; Respiratory Sensitizer. NRS; Non-respiratory sensitizer. Green and red indicates correct classifications and incorrect classifications, respectively.

FUNCTIONAL & MECHANISTIC RELEVANCE

Estimating the predictive performance of GARDair based on the available data, the predictive accuracy was calculated to 89%, well-balanced between sensitivity and specificity. Furthermore, based on the few repeated exposures available from independent experiments, the reproducibility was 100%, indicative of a robust assay. Thus, the assay is demonstrated to be functional and predictive, capable of accurate identification of respiratory sensitizers from both true non-sensitizers and skin sensitizers.

It is hypothesized that the specific recognition of respiratory sensitizers in a DC model *in vitro* is associated with the decision-making role of DCs and the induction and modulation of downstream events in the immunological cascade. Specifically, the assay is proposed to monitor transcriptional changes in DCs, as induced specifically by respiratory sensitizers, related to the activation of DCs, bridging of innate and adaptive immune functions and skewing towards Th2 type immune responses.

CONCLUSION

GARDair is a novel assay for assessment of respiratory sensitizers. It is an adaptation of the GARD platform, utilizing gene expression analysis of predictive biomarker signatures and state-of-the-art data analysis methodology. GARDair has been proven functional and is currently progressing towards industrial implementation and regulatory acceptance with financial support from the EU programme Horizon 2020. This progress includes scientific verification of results, assay optimization, assay transfer and formal validation by a blinded ring trial.

Acknowledgement
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Additional reading
Forreryd A and Johansson H et al. ***Prediction of Chemical Respiratory Sensitizers Using GARD, a Novel In vitro Assay Based on a Genomic Biomarker Signature.*** PLOS ONE. 2015
Forreryd A et al. ***Evaluation of high throughput gene expression platforms using a genomic biomarker signature for prediction of skin sensitization.*** BMC Genomics. 2014

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