Prediction of chemical respiratory sensitizers using GARD

a novel in vitro assay based on a genomic biomarker signature

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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.

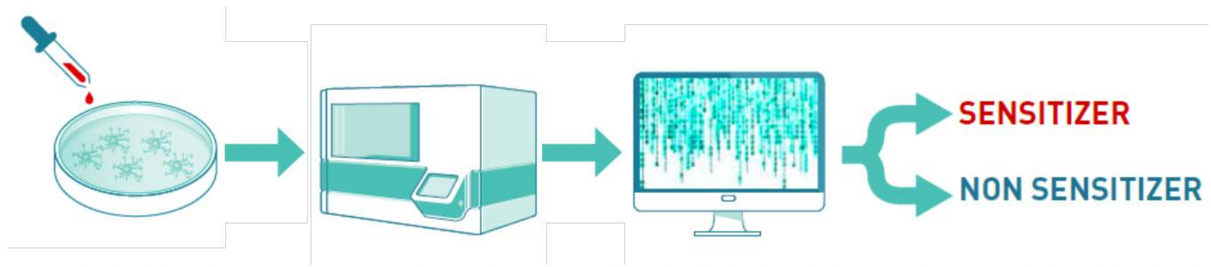

Figure 1. GARD – Genomic Allergen Rapid Detection. Chemically-induced changes in transcriptional levels of cells exposed to test chemicals are compared to predictive genomic biomarker signatures using supervised machine learning. Unknown samples are classified as either skin sensitizers, respiratory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

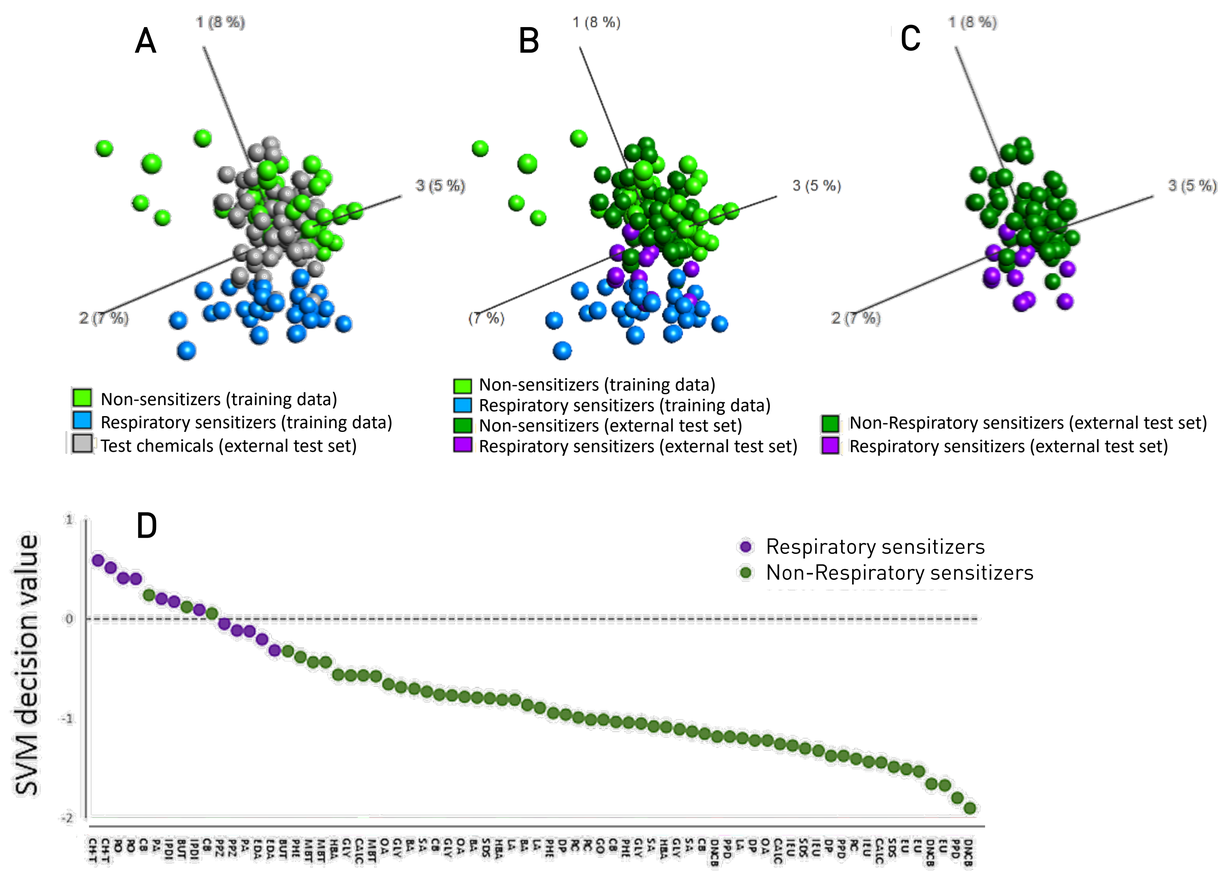

Figure 3. Visualization of the discriminatory capabilities of the respiratory sensitizer-specific biomarker prediction signature and subsequent classification of an external test set using principal component analysis (PCA). (**A**) The panel of reference chemicals used to identify the GRPS (training 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.

RECENT DEVELOPMENT

Following scientific proof-of-concept studies, the GARDair concept has been further developed and is progressing towards industrial implementation. In a project spanning over two years, funded by the EU Framework Programme for Research and Innovation Horizon 2020, the technology is moving through phases of reconfirmation of scientific results, assay optimization, assay transfer and validation and exploitation of results, through a directed service launch and offering to the market (Figure 3).

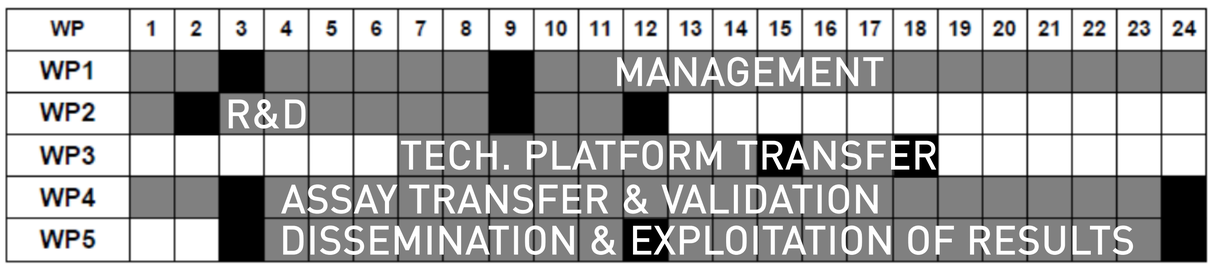

Figure 3. Schematic overview of project implementation across different work packages. Expected dates of deliverables are highlighted. Project start (Month 1) was the 1st of March, 2017.

In the first phase of R&D (WP2), the validity of initial scientific results have been confirmed. The GRPS was optimized and condensed to a set of 29 genes, which 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 (WP3). Following model and assay optimization and the formalization and review of assay procedures in a Standard Operating Proceedure (SOP), the GARDair assay is currently being readied for a blinded validation study (WP4) (Figure 4).

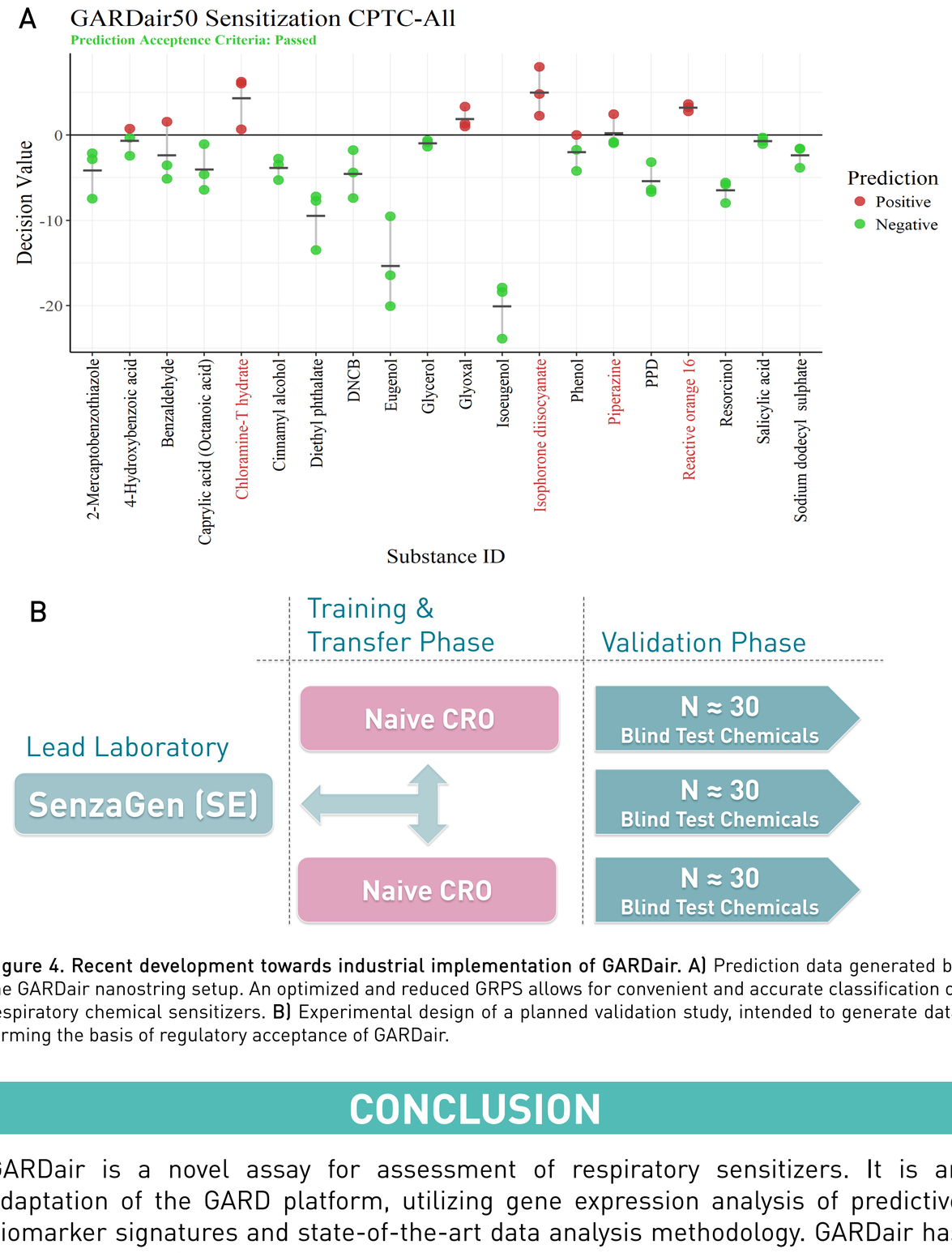

Figure 4. Recent development towards industrial implementation of GARDair. A) Prediction data generated by the GARDair nanostring setup. An optimized and reduced GRPS allows for convenient and accurate classification of respiratory chemical sensitizers. **B)** Experimental design of a planned validation study, intended to generate data forming the basis of regulatory acceptance of GARDair.

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 with financial support from the EU programme Horizon 2020. This progress will include scientific verification of results, assay optimization, transfer and formal validation.

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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