Inter-laboratory ring trial of the GARD™air assay for assessment of respiratory sensitizers

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Conclusions

- GARDair is a novel *in vitro* assay for assessment of respiratory sensitizers, based on evaluation of exposure-induced gene expression of genomic biomarkers in a Dendritic Cell-like cell and machine-learning assisted classification.
- GARDair is the first in its class to be subjected to an inter-laboratory ring trial
- The method is highly specific, with high PPV, making it suitable for opt-out applications within product development and candidate prioritization.

Introduction

Sensitization of the respiratory tract by sensitizing chemicals may lead to severe bronchoconstriction and asthma-like symptoms with a potentially fatal outcome. However, proactive identification and characterization of respiratory sensitization hazards is currently hampered by the lack of validated or even widely used predictive assays.

The GARD™ platform utilizes exposure-induced gene expression profiles of a dendritic cell-like cell line in combination with machine learning as a basis for predictive *in vitro* assays for different immunotoxicity endpoints (Johansson et al., 2020). Functional evidence of the ability to accurately identify respiratory sensitizers using the GARD platform has previously been demonstrated (Forreryd et al., 2015). Here, we present GARDair, an adaptation of previous findings on a gene expression analysis platform suited for routine acquisition, based on an optimized predictive biomarker signature. Furthermore, we present the initial results of an inter-laboratory ring-trial, demonstrating the functionality, transferability and reproducibility of the assay.

Accuracy	Sensitivity	Specificity	PPV	NPV	BLR	WLR
74%	53%	95%	92%	66%	79%	52-72%

Table 1. Key performance statistics from the GARDair inter-laboratory ring trial. PPV; Positive Predictive Value. NPV; Negative Predicitve Value. BLR; Between Lab Reproducibility. WLR; Within Lab Reproducibility

References

Johansson et al., 2020. Genomic Allergen Rapid DetectionTM – A modular testing strategy framework for sensitization hazard and risk assessment. Toxicology in vitro. In review.

Forreryd et al., 2015. Prediction of chemical Respiratory sensitizers using GARD, a novel *in vitro* assay based on a genomic biomarker signature. PLOS ONE.

Results

An optimized training dataset was constructed from repeated experiments, reproducing previous findings in a NanosString nCounter® format (Figure 1). Following *de novo* analyses, a finalized genomic biomarker prediction signature was established, consisting of 28 genomic predictors. Evaluation of transcriptional patterns of the finalized prediction signature allows for differentiation between respiratory sensitizers and non-respiratory sensitizers.

A prediction model based on Support Vector Machines was defined, the appropriate utilization of which were described in assay protocols. As such, the GARDair assay was defined and frozen prior to being subjected to an inter-laboratory ring trial following assay transfer to naïve laboratories.

The ring trial comprised blind assessment of 29 compounds, including expected respiratory sensitizers, skin sensitizers and non-sensitizers. Results are presented in Figure 2, with key performance statistics summarized in Table 1.

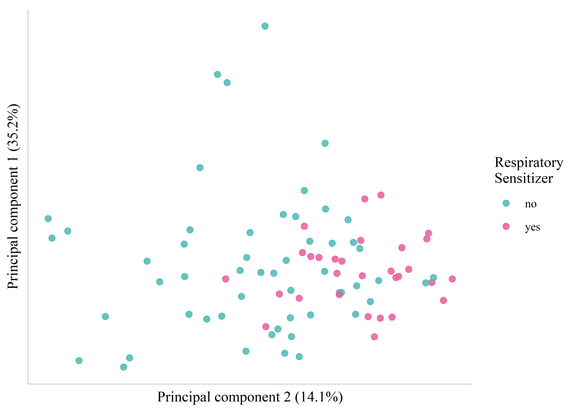

Figure 1. Unsupervised representation of the GARDair training dataset using Principal Component Analysis.

Discussion

Respiratory sensitization is a toxicological endpoint to which predictive assays are currently lacking. Here, we present GARDair, a first-in-class assay based on genomic biomarkers evaluated in Dendritic Cells. The genomic biomarker signature monitors cellular events associated with Th2-differentiation, allowing for accurate and specific differentiation from e.g. skin sensitizers, as demonstrated. Furthermore, a ring trial aimed at evaluating assay performance was performed. It was shown that the assay is transferable and highly specific, thus optimal for opt-out applications in chemical and pharmaceutical industry for efficient and resource-effective prioritization among candidates based on safety profiles. For regulatory purposes, we propose that GARDair may contribute valuable information to weight-of-evidence approaches, as currently moderate levels of sensitivity, primarily in isocyanate and anhydride chemical subsets, advocates the integration of complementary sources of information, such as *in silico* structure-alerts and (Q)SAR methodologies. In conclusion, GARDair exhibits unique properties in specific hazard identification of respiratory sensitizers and constitutes significant progress towards accurate safety assessment of chemicals.

Non-respiratory sensitizer

Respiratory sensitizer

Figure 2. GARDair inter-laboratory ring trial results. Classifications represent the majority outcome of three independent experiments from each laboratory.

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