

Andy Forreryd<sup>1</sup>, Joshua Vaughan<sup>2</sup>, Michelle Hernandez<sup>2</sup>, Olivia Larne<sup>1</sup>, Robin Gradin<sup>1</sup>, Henrik Johansson<sup>1</sup>.  
<sup>1</sup>SenzaGen AB, Lund, Sweden. <sup>2</sup>Merck & Co., Inc., Rahway NJ, USA

## Summary

- GARD<sup>air</sup> is an experimental *in vitro* assay for assessment of respiratory sensitizers, based on evaluation of exposure-induced gene expression changes of genomic biomarkers in a Dendritic Cell-like cell line and machine learning-assisted classifications.
- GARD<sup>air</sup> has previously been shown to be functional, having been subjected to a validating ring trial, demonstrating its capability to predict respiratory sensitization properties of low-molecular weight test chemicals.
- Here, we investigated the hypothesis that protein allergens engage similar toxicity pathways as low-molecular weight chemicals and demonstrated that GARD<sup>air</sup> can be successfully used to predict the respiratory sensitizing properties of the model allergen Subtilisin.

## Introduction

Sensitization is a condition induced by an immune system response to a variety of molecules, including proteins or chemicals, referred to as sensitizers. Proactive identification of sensitizers is central in hazard and risk assessment of both biologics and chemicals, for regulatory registration or to ensure occupational safety. While large investments in New Approach Methodologies for assessment of dermal sensitizers have been made, the ability to accurately predict respiratory sensitizers *in vitro*, including both low-molecular weight chemicals and biologics such as proteins and peptides, remains unfulfilled.

The Genomic Allergen Rapid Detection assay for hazard assessment of respiratory sensitizers (GARD<sup>air</sup>) is an experimental method, originally developed to provide binary hazard identification of chemical respiratory sensitizers. Based on the same technological framework as the OECD validated GARD<sup>skin</sup> assay (OECD TG 442E), the method evaluates the transcriptional patterns of disease-associated genes in the dendritic cell-like cell line SenzaCell<sup>TM</sup>, following test item exposure. Here, we hypothesized similar toxicity pathway engagement by protein sensitizers as for low-molecular weight chemicals. Using the model protein allergen Subtilisin, a well-known inducer of occupational asthma, we here demonstrate the ability of GARD<sup>air</sup> to assess the respiratory sensitization potential of proteins.

## Methods

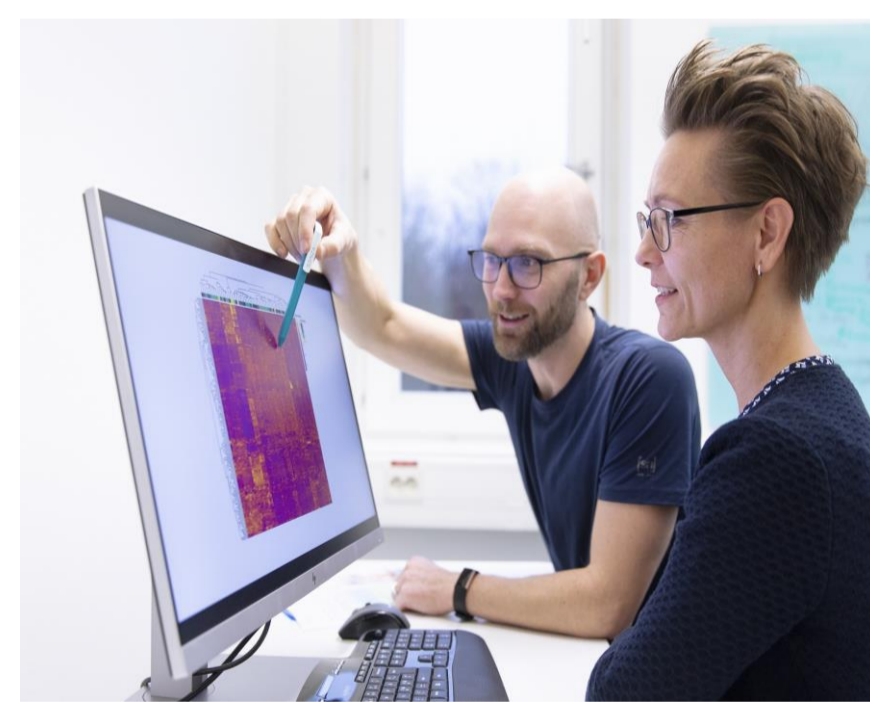
- The GARD<sup>air</sup> method is based on transcriptomic measurements of a set of genomic biomarkers, referred to as the GARD<sup>air</sup> Genomic Prediction Signature (GPS) in the SenzaCell DC-based cell line. An overview of the protocol is presented in Figure 1.



**Step 1**  
Expose Cells (the SenzaCell<sup>TM</sup> cell line) to the test sample at determined concentration.



**Step 2**  
Measure the gene expression levels of 28 biomarkers, the genomic biomarker signature.



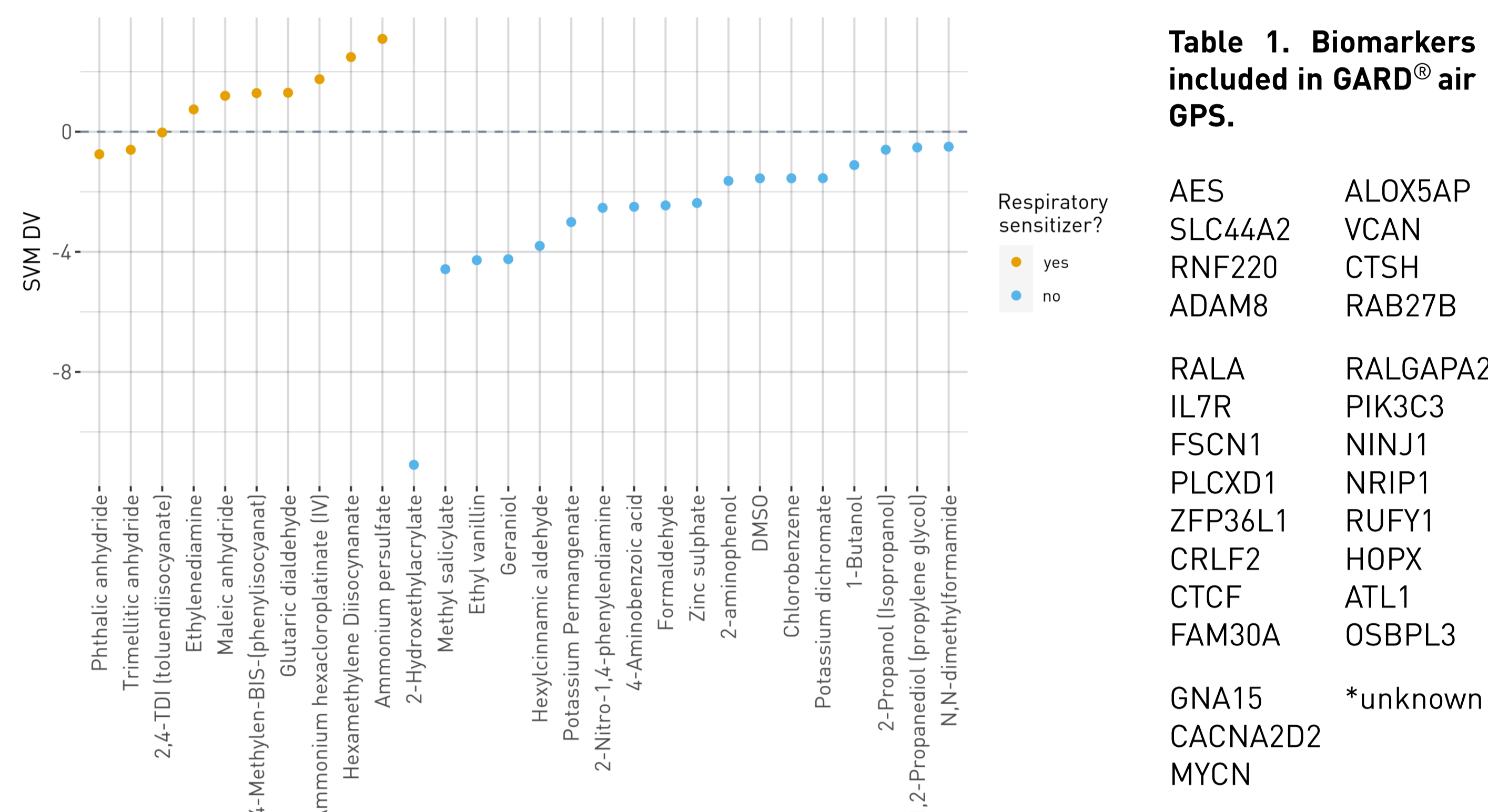
**Step 3**  
GARD<sup>air</sup> Data Analysis Application makes a binary prediction based on gene expression analysis.

### Figure 1. GARD<sup>air</sup> protocol in three steps

Testing of the protein was performed according to the GARD<sup>air</sup> protocol. In short: **(Step 1)** Cells were exposed to test item under 24h. **(Step 2)** Total RNA were isolated from the cells, and the gene expression of the 28 genes in the GARD<sup>air</sup> Prediction Signature was measured. **(Step 3)** Gene expression data were uploaded into the cloud-based GARD<sup>air</sup> Data Analysis Application (GDAA) harbouring the data analysis pipeline, including the Support Vector Machine (SVM) based prediction model.

- The GPS was defined using an optimized training dataset constructed from repeated exposures of a well-characterized reference set of chemicals (respiratory sensitizers and non-respiratory sensitizers). A data-driven approach, including state-of-the-art statistical methods, established the GARD<sup>air</sup> GPS comprising 28 genes (Table 1).
- The potential of the genes in the GARD<sup>air</sup> GPS to discriminate respiratory sensitizers from non-respiratory sensitizers are illustrated in Figure 2.

- In the present study, the GARD<sup>air</sup> method was used to predict the sensitizing properties of Subtilisin, a well-characterized respiratory allergenic protein.
- In short, the SenzaCell cell line was used as an *in vitro* model for dendritic cells (DC). Cells were exposed to the test item Subtilisin (25µg/ml) along with relevant negative (vehicle) and positive controls.

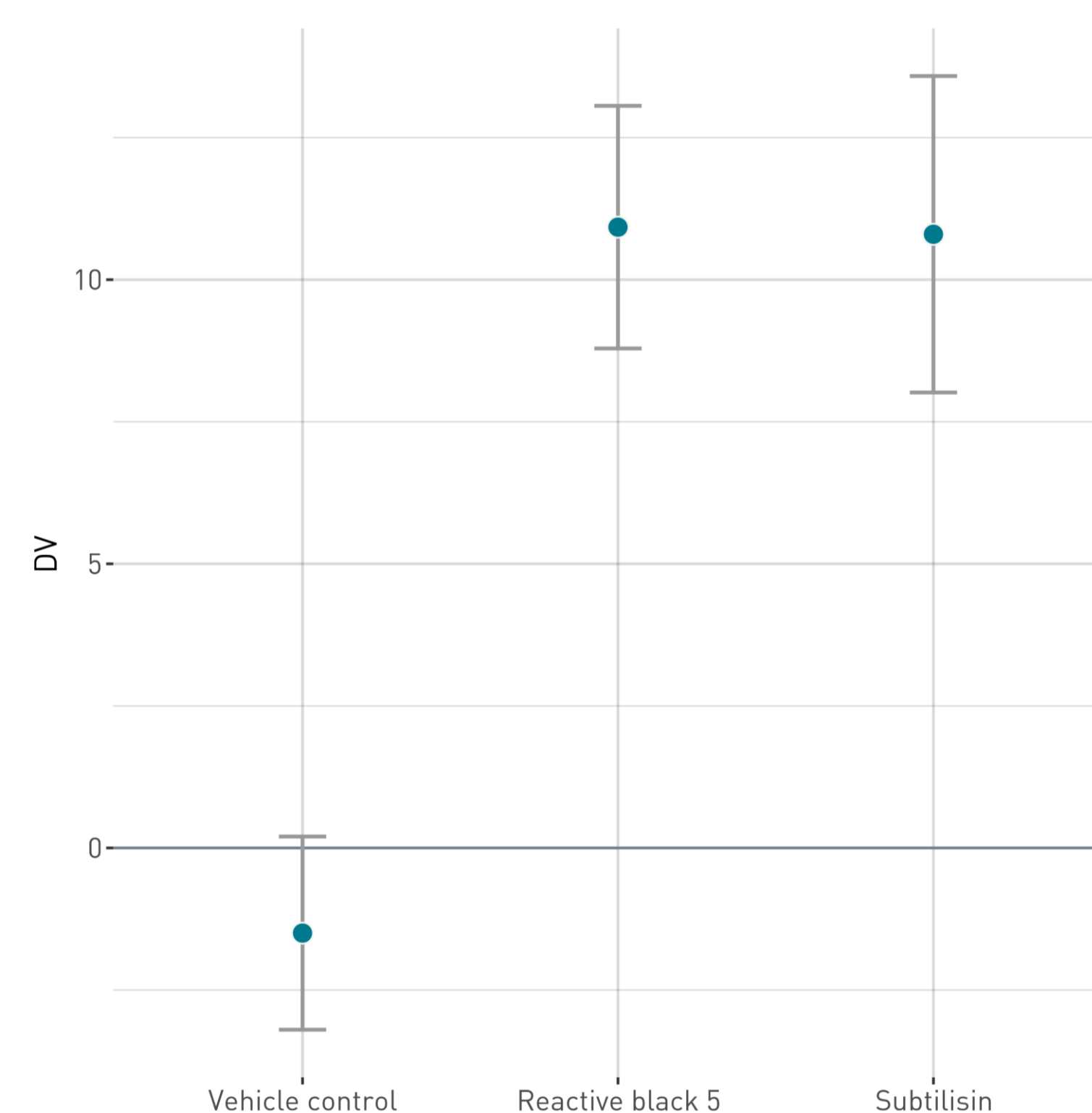


**Figure 2. SVM DVs for test items in the training dataset.**

The figure represents the mean distances between the training sets gene expression patterns and the prediction models classification border, which is used to classify novel test items as sensitizers (positive DVs) or non-sensitizers (negative DVs) based on their induced gene expression levels. As such, chemicals with relatively larger distances to the classification border may be seen as having an effect on the cell system that makes them relatively easier to distinguish.

## Results & Discussion

The GARD<sup>air</sup> test results of the Subtilisin and control samples are presented in Figure 3. Subtilisin gives rise to clearly positive response signals, along with the low-molecular weight positive control (Reactive black 5), while the negative (vehicle) control does not. Evidently, the protein allergen Subtilisin triggers, at least to a limited albeit sufficient extent, similar toxicity pathways as the low-molecular weight chemical space used to define the method. While these findings are solely originating from an *in vitro* setting, we hypothesize that such toxicity pathways would be similarly engaged also in an *in vivo* setting, thereby, at least partly, explaining the allergenic effects known to be associated with Subtilisin exposure. Further, we hypothesize that such toxicity pathways would, at least partly, be associated with the GARD<sup>air</sup> GPS, which is proposed to monitor transcriptional changes induced specifically by respiratory sensitizers, related to the bridging of innate and adaptive immune functions and skewing towards Th2 type immune responses (including TSLP and IL-7R-alpha chain).



**Figure 3. GARD<sup>air</sup> results generated within the study.** Figure shows the mean GARD<sup>air</sup> DV from replicates (n=3). Mean DV > 0 is classified as respiratory sensitizer. The vehicle control and the positive control were correctly classified as non-respiratory sensitizer and sensitizer, respectively. The test item Subtilisin was classified as a respiratory allergen.

## Conclusions

- Based on the findings reported in this poster, GARD<sup>air</sup> may constitute a valuable tool for assessment of respiratory sensitization properties of chemicals and proteins.
- Given the limitation of this study with respect to sample size, next steps include the generation of more data to support the predictive capacity, including the assessment of proteins with low (or lack of) sensitization potential.

## Reference

For a conceptual overview of GARDair and respiratory sensitization, please see the following publication:  
 Forreryd et al., Prediction of chemical Respiratory sensitizers using GARD, a novel *in vitro* assay based on a genomic biomarker signature. PLoS One 2015 Mar

## Contacts

Henrik Johansson, PhD | henrik.johansson@senzagen.com  
 Andy Forreryd, PhD | andy.forreryd@senzagen.com