Exploration of the GARD® applicability domain - Sensitization assessment of UVCBs

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Introduction

The GARD® – Genomic Allergen Rapid Detection – platform is a state of the art *in vitro* assay for assessment of chemical sensitizers. The GARD®skin assay is a powerful tool for assessment of chemical sensitizers, with a predictive accuracy of 94%. In this study, four UVCB test items, provided by Lubrizol and selected based on existing *in vivo* data (internal Lubrizol data), were evaluated. Sensitizing hazard was assessed using the GARD®skin assay, and the GARD®potency assay further subcategorized the sensitizers into strong (1A) or weak (1B) sensitizers according to GHS/CLP classification. Here we show the importance of using appropriate vehicles in order to predict a correct classification of Test items.

Conclusion

A UVCB Test item with poor water and DMSO solubility was assessed using a mixture of vehicles with different polarity indexes (DMF and Glycerol 1:1). This experimental vehicle mixture classified the UVCB as a skin sensitizer, being consistent with the *in vivo* data. This case study demonstrates the broadening of the applicability domain of the GARD®assay when assessing UVCBs.

The GARD platform

Human immunologically relevant cells are used

The cells are exposed to the substance of interest

Their genomic products (transcripts) are isolated

The gene transcripts are quantified
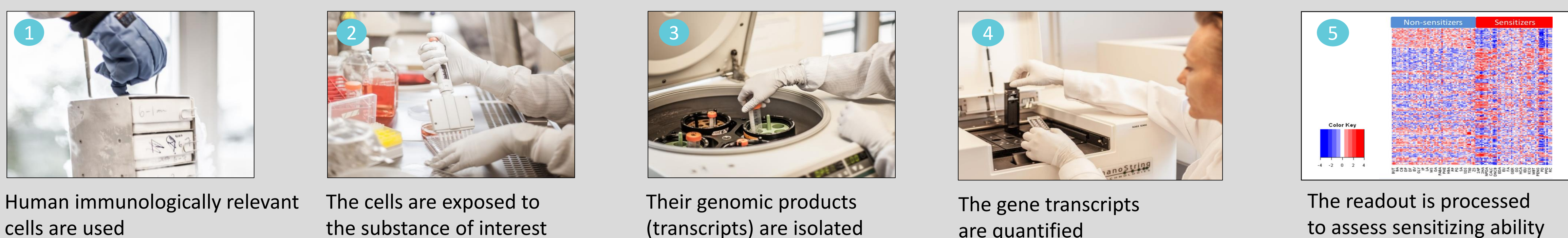
The readout is processed to assess sensitizing ability

Figure 1. The dendritic cells (DCs) are sentinels of the immune system during sensitization. The GARD®platform is based on SenzaCells, a human myeloid cell line similar to DCs. The assay protocol includes stimulation of the cells by the test items and after incubation the transcripts are harvested. The NanoString technology is used for quantification of GARD®skin and GARD®potency signatures, 200 and 52 genes respectively. Bioinformatics multivariate technology is used for gene expression analysis.

Method

Four Test items of "Unknown or Variable composition, Complex reaction products and Biological materials" (UVCB) were analysed according to the GARD®skin and GARD®potency assays. Test items 2 – 4 were assessed according to the GARD®SOP. For Test item 1, being insoluble in the standard GARD®assay solvents, an extraction procedure was performed with DMSO. In parallel, a mixture of two vehicles with different polarity indexes (DMF:Glycerol 1:1, DMF:H_2O 1:3, 1:1 and 3:1 and Glycerol:H_2O 1:3, 1:1 and 3:1) known to be non-sensitizers in the GARD®skin assay, was explored. For the full procedure of the GARD®skin assay, see Johansson *et al.* ALTEX, 2017 and for GARD® potency, see Zeller *et al.* ALTEX, 2017.

Result

When using DMSO as a vehicle, three of the four Test items (Test items 2 – 4) were predicted as sensitizers and classified as 1B according to GHS/CLP classification (Table 1). For Test item 1, depending on vehicle used, discrepant pre-dictions were seen. When using the DMSO extraction procedure, Test item 1 was predicted as a non-sensitizer and a potency classification as No Cat (Table 1). However, when dissolved in DMF and Glycerol the Test item 1 was classified as a sensitizer (Table 1 and Figure 2). The GARD®skin predictions for Test items 1 (DMF/Glycerol), 2 and 3 and the GARD®potency classifications for Test items 2 and 3 were consistent with the *in vivo* data, whereas Test item 4 showed inconsistency between the *in vitro* and *in vivo* methods.

Table 1. GARD®skin prediction, GARD®potency and *in vivo* classification

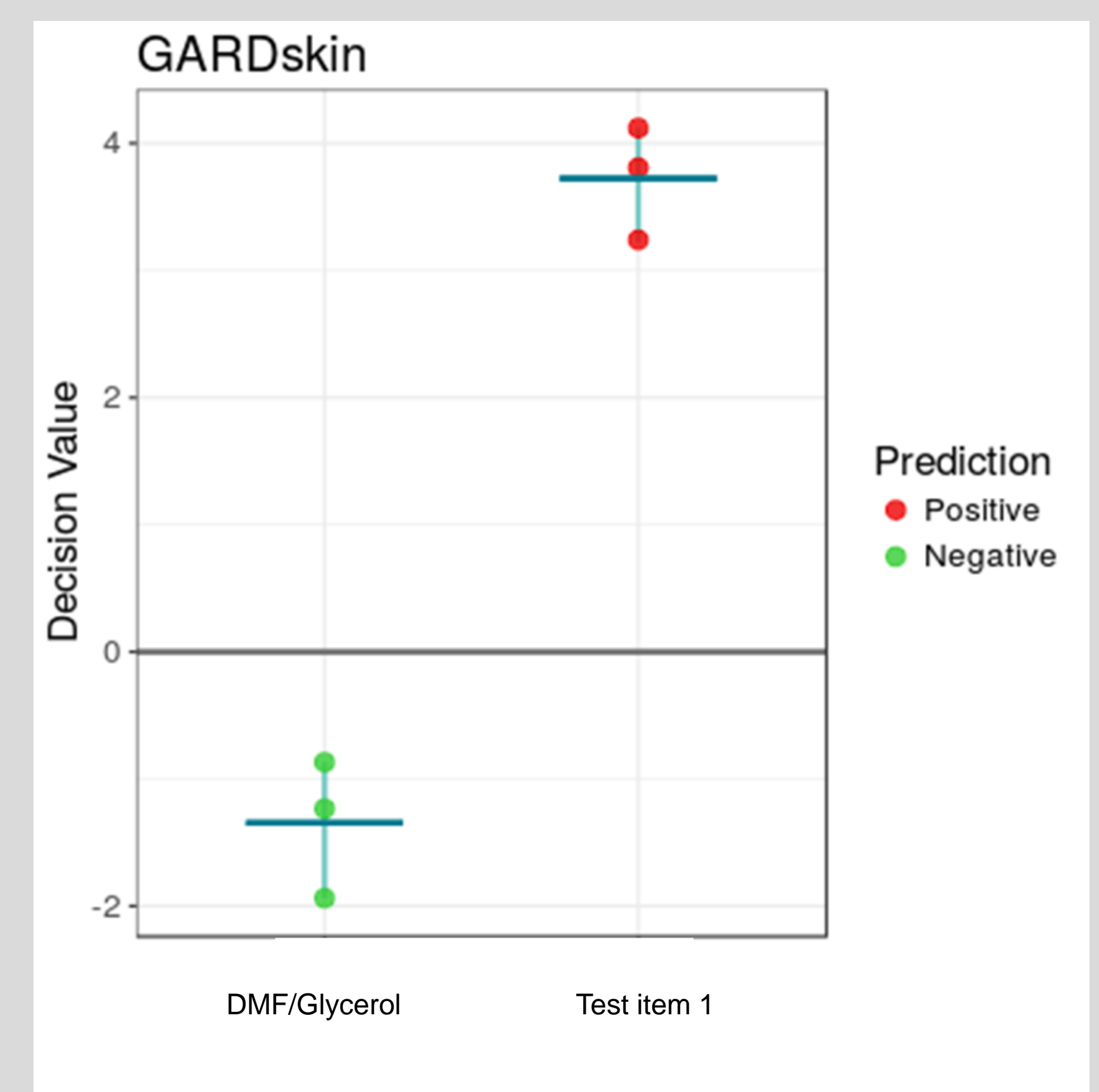| Test items	Vehicle	GARD®skin	GARD®potency	*In vivo*
Test item 1	DMSO	Non-sens	No Cat	1B
	DMF/Glycerol	Sens	Not assessed	1B
Test item 2	DMSO	Sens	1B	1B
Test item 3	DMSO	Sens	1B	1B
Test item 4	DMSO	Sens	1B	Non-sens
Pos ctrl (PPD)	DMSO	Sens	1A	Not assessed
Neg ctrl	DMSO	Non-sens	Non-sens	Not assessed
Neg ctrl	DMF/Glycerol	Non-sens	Not assessed	Not assessed

Figure 2. GARD®skin prediction of Test item 1 and corresponding vehicle mixture, DMF/Glycerol

Discussion

These results indicate the importance of screening a panel of different vehicles, or mixtures thereof, in order to choose the appropriate solvent. For one of the Test items, the DMSO extraction procedure generates a negative prediction while the experimental vehicle mixture, Glycerol and DMF, classifies the chemical as a skin sensitizer. This case study demonstrates the broadening in applicability domain of the GARD®assay when assessing UVCBs.

References:
Johansson *et al.* ALTEX, 2017| Johansson *et al.* BMC Genomics, 2011| Forreryd *et al.* BMC Genomics, 2014| Johansson *et al.* Toxicol Sci, 2014| Zeller *et al* ALTEX, 2017|

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