The GARD™potency assay for potency-associated subclassification of chemical skin sensitizers – rationale, method development and ring trial results of predictive performance and reproducibility

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Introduction

Proactive identification and characterization of sensitization hazards are central aspects of risk assessment of chemicals. Current legislations and trends in predictive toxicology advocate a transition from *in vivo* methods to non-animal alternatives. While validated methods for skin sensitization hazard are available, non-animal methods capable of supporting Classification, Labelling and Packaging (CLP) sub-categorization are still lacking.

Methods

The GARD platform utilizes exposure-induced gene expression profiles of a dendritic cell-like cell line in combination with machine learning to provide hazard classifications for different immunotoxicity endpoints. The GARDskin assay for hazard assessment of skin sensitizers has been validated in a blinded ring trial, exhibiting high levels of predictive performance and reproducibility. More recently, a novel predictive genomic biomarker signature for potency-associated discrimination between weak and strong skin sensitizers was proposed.

Here, we present the adaptation of the defined biomarker signature on a gene expression analysis platform suited for routine acquisition and confirm the validity of the proposed biomarkers. Following prediction model establishment, the GARDpotency assay for discrimination of strong (1A) sensitizers from weak (1B) sensitizers and non-sensitizers was defined (Figure 1). Furthermore, a GARD Defined Approach (DA), consisting of a sequential combination of GARDskin and GARDpotency for complete assessment and sub-categorization of skin sensitizers, was defined. The performance of GARDpotency and the GARD Defined Approach was evaluated in a blinded ring-trial by assessing predictive performance and reproducibility.

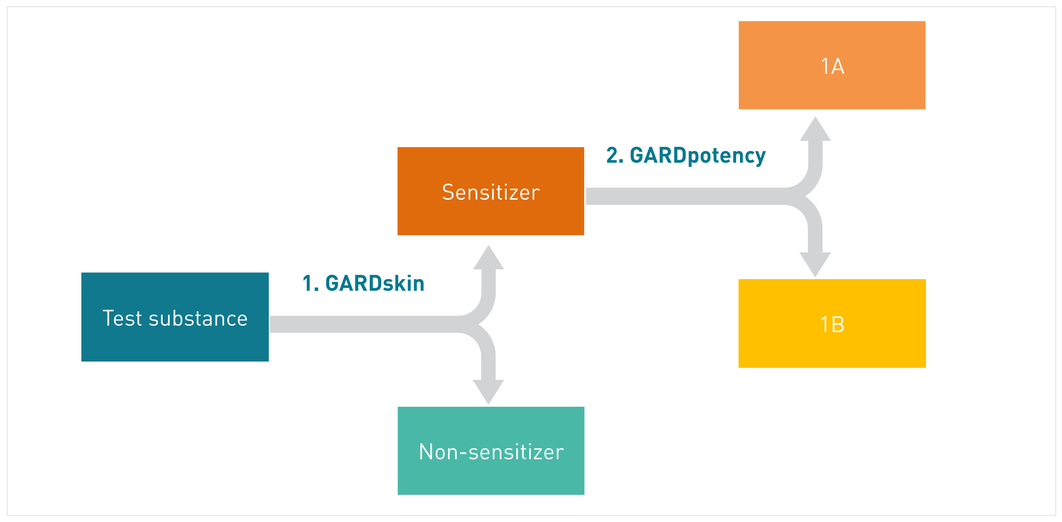

Figure 1. Sequential combination of the GARDskin and GARDpotency assays forms the GARD Defined Approach, allowing for combined hazard assessment and CLP sub-categorization of skin sensitizers.

Results

Following genome-wide gene expression analysis of exposure-induced transcriptional patterns, a genomic biomarker signature predictive for sensitizing potency was identified (Zeller et al., 2017). It was shown that discriminatory capabilities were maintained in a NanoString nCounter format (Figure 2A). Furthermore, it was shown that predictive information was provided by the GARD input concentration parameter (Figure 2B). As such, a finalized GARDpotency prediction model was defined, based on these measurable parameters.

The finalized GARDpotency assay was transferred to naïve laboratories and subjected to a blinded ring trial for formal validation and regulatory acceptance (Gradin et al., 2020). In summary, the predictive accuracy of GARDpotency was estimated to 91% (Table 1).

Furthermore, when combined with GARDskin (Johansson et al., 2019), the GARD Defined Approach for complete hazard and risk assessment of chemicals into three categories demonstrated an estimated predictive accuracy of 86% (Figure 3).

Figure 2. A) Genomic biomarkers provide predictive information related to sensitizing potency, as illustrated by Principal Component Analysis of the training dataset. B) The GARD input concentration with which individual test items are assayed provide predictive information related to sensitizing potency, as illustrated by the concentrations used for assessment of chemical constituents of the training dataset.

Conclusion

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- GARDpotency is an assay for sub-categorization of strong sensitizers (CLP sub-category 1A), allowing for discrimination from weak sensitizers (CLP sub-category 1B) and non-sensitizers. The method is based on the GARD platform, combining human immune cells, a genomic biomarker readout and machine learning-assisted classifications.
- Sequential combination of GARDskin and GARDpotency forms the GARD Defined Approach, for complete hazard and risk assessment of skin sensitizers into three categories (CLP 1A, CLP 1B, non-sensitizers).
- A blinded ring trial, comprising 28 chemicals, demonstrated that GARDpotency is functional and reproducible, with an accumulated predictive accuracy of 91% across three laboratories. In the same dataset, the GARD Defined Approach classifies chemicals into three categories with 86% accuracy.

Table 1. Predictive performance of the GARDpotency assay. Estimations of predictive accuracy and class-specific sensitivity, as obtained in a ring trial comprising three independent laboratories. Individual results for each laboratory, as well as accumulated performance, are reported by contingency tables and calculations of Cooper statistics.

Expected value	Predictions (BRT)		Predictions (Eurofins)		Predictions (SenzaGen)		Predictions (Accumulated)	
	1A	1B	1A	1B	1A	1B	1A	1B
1A	8	1	10	1	10	1	28	3
1B	0	15	0	16	4	13	4	41
Accuracy (%):	95.8		96.2		80.8		91.1	
Specificity (%):	88.9		94.4		94.4		90.3	
Sensitivity (%):	100		100		76.5		91.7	

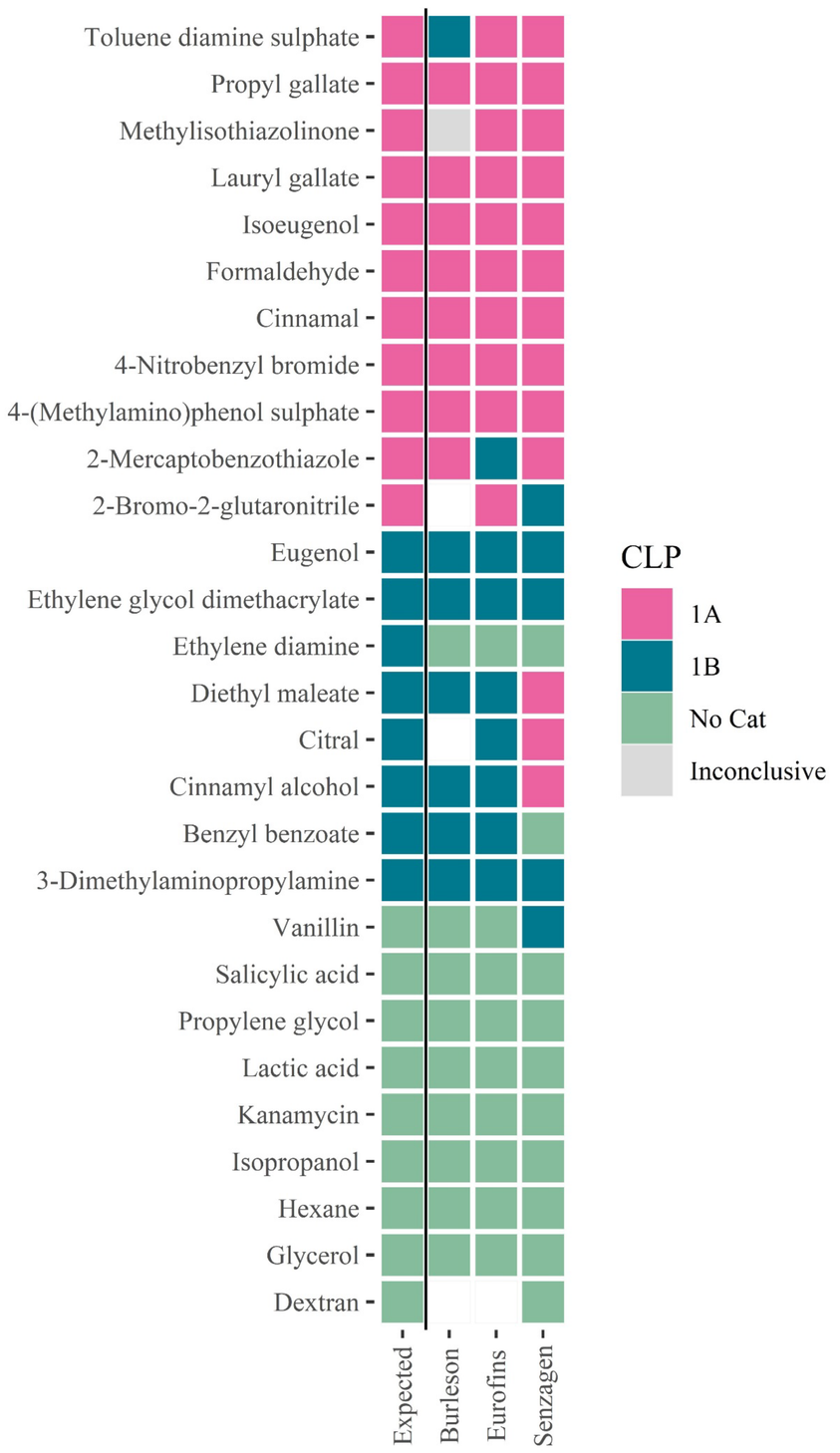

Figure 3. GARD Defined Approach results, as obtained from a blinded ring trial for method validation.

References

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