GARD® – The Story

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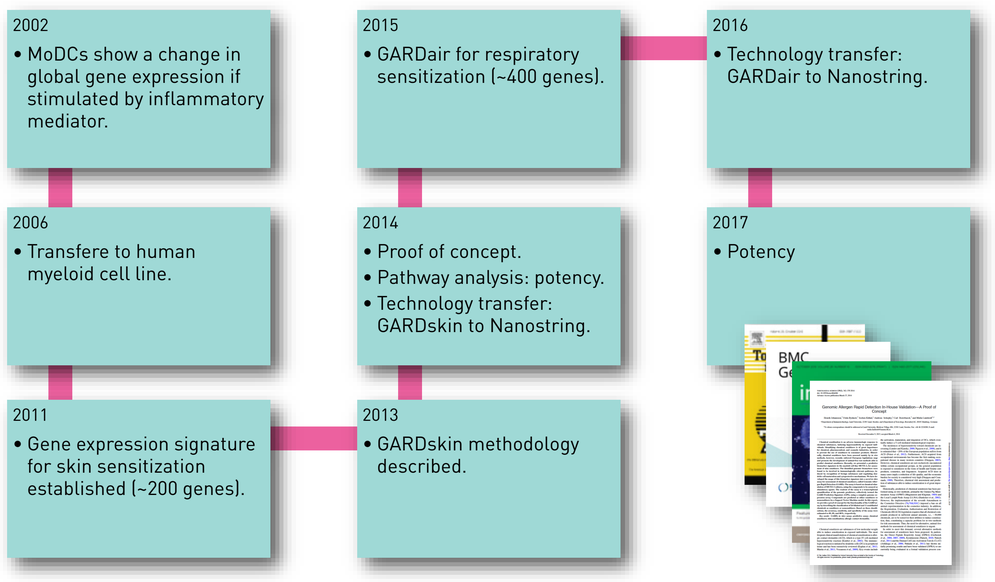
BACKGROUND

To prevent the general population for unnecessary exposure to sensitizing substances, the substances have to be safety tested. Regulatory authorities and economic interests request animal free methodology. Genomic Allergen Rapid Detection, **GARD**, is an *in vitro* test developed for the prediction of sensitizing chemicals. It is based on differential expression of disease-associated genomic biomarkers in a human myeloid dendritic cell line.

Here, we describe the development of the **GARD** platform and its downstream innovations.

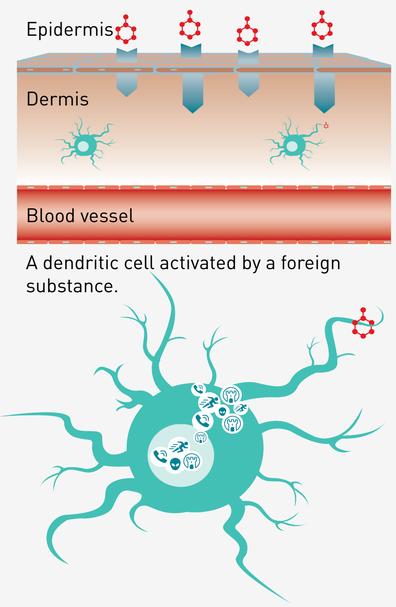
RESUME



THE SCIENCE BEHIND

In vivo, dendritic cells (DCs) are activated by molecules secreted from surrounding cells stimulated by foreign substances or directly by the foreign substance (1). The following alteration of the DC proteome facilitates a multitude of cellular modifications leading to cell maturation, which in its final step connects the innate and adaptive immune system.

The foundation of **GARD** was established in 2002 (2): it was revealed that if stimulated by inflammatory mediators *in vitro*, dendritic cells (DCs) derived from blood, change their global gene expression similar to DCs *in vivo*. To circumvent donor variability and to be able to expand the research, the work was later transferred from primary cells to a human myeloid cell line with characteristics similar to human DCs (3).



A dendritic cell activated by a foreign substance.

In vivo, the DCs can recognize foreign substances and become activated to signal self-defence to other parts of the immune system. The GARD assay mimics the *in vivo* system *in vitro* and by measuring changes in gene expression chemical's sensitization properties can be predicted.

Stimulating the cell line with 40 chemicals (20 non-sensitizers and 20 sensitizers) a genomic biomarker signature predicting skin sensitization was identified through gene expression analysis of the transcriptome (4). The signature level is the end-point measurement in **GARDskin** (5) and the predictive signature consists of ~200 genes discriminating sensitizers from non-sensitizers with higher accuracy (~90%) compared to the traditional murine Local Lymph Node Assay (LLNA, 70-75%). The functionality of **GARDskin** was confirmed in a proof of concept study, where 26 blinded and 11 non-blinded chemicals were analyzed(6).

While discovery studies were performed using microarray technology, alternative platforms have been evaluated, in order to better suit industrial and regulatory screening procedures. Today, the Nanostring method is utilized composing a streamlined, robust, cost-effective platform, adequate for the industrial high throughout demand (7, 8).

THE GARD PLATFORM

Cells are stimulated with the test substance and after incubation mRNA is isolated and quantified using Nanostring technology. The final prediction call (classification) is derived from multivariate machine learning techniques and pattern recognition (5).

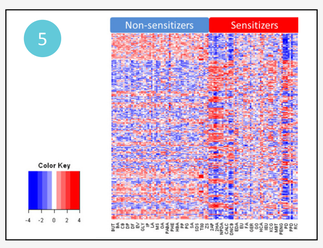
A brief overview of the GARD process

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.

Final report.

FURTHER INNOVATIONS

Depending on requested end-point, the **GARD** assay is flexible. The original assay, **GARDskin**, was developed for assessing skin sensitizers. Likewise, **GARDair** for assessing respiratory sensitization, is based on the **GARD** platform, and the genomic biomarker signature consists of ~400 genes, accuracy ~85% (9).

By a bioinformatic pathway analysis of the **GARDskin** gene expression profile, it was shown that the profile can be used for potency classification (10). This work is an ongoing process and further refinements show good results (manuscript in preparation).

VALIDATION

GARDskin was included in the OECD test guide line programme (TGP no. 4.106), spring 2016, and an ECVAM ring trial is set up at two external test laboratories to confirm the reproducibility and transferability. A method evaluation on chemicals provided by Cosmetics Europe Skin Task Force has been executed and the results are confirming that accuracies are consistently high (manuscript in preparation).

References: 1. Johansson *et al.* Basic Clin Pharmacol Toxicol, 2014. **2.** Lindstedt *et al.* Int Immunol, 2002. **3.** Larsson *et al.* Immunology, 2006. **4.** Johansson *et al.* BMC Genomics, 2011. **5.** Johansson *et al.* Toxicol In Vitro, 2013. **6.** Johansson *et al.* Toxicol Sci, 2014. **7.** Forreryd *et al.* BMC Genomics, 2014. **8.** Forreryd *et al.* Toxicol In Vitro, 2016 **9.** Forreryd *et al.* PLoS One, 2015. **10.** Albrekt *et al.* BMC Pharmacol Toxicol, 2014.