In Vitro Skin Sensitization Testing – U-SENS™ Assay

Need

Assessing the risk of dermal sensitization is a primary consideration in determining the safety of a dermatological drug or cosmetic. It is also of concern in the case of accidental exposures to chemicals, formulations, and other products. Skin sensitization is a complex chemical and biological mechanism, which has been summarized as an Adverse Outcome Pathway (AOP; OECD 168). The third key event in skin sensitization is the activation of dendritic cells.

For new dermatological products and substances that may come in contact with human skin, the cost and time necessary to assess dermal sensitization *in vivo* is prohibitory to efficient product development and safety assessment. Assessment of sensitization potential has historically been performed using animal models, however, the anatomy and barrier physiology of research animal model skin, including hair follicle density and stratum corneum and viable epidermis thickness, do not accurately mimic that of human skin.

Solution

The effect of dermatological product formulation on dermal sensitization should be determined in early *in vitro* efficacy studies. Dermal sensitization can be determined by using non-animal *in vitro* methods, more specifically, using three separate assays, each corresponding to one of the first three key events in the AOP. One of the assays which assesses the third key event, activation of dendritic cells, is the U-SENS assay, performed under OECD Test Guideline 442E. This assay uses U937 cells, which are a pro-monocytic model cell line. Upon activation of the U937 cells, an increase in expression of CD86 is observed, a hallmark of dendritic cell and immune system activation, and a necessary costimulatory molecule for T-cells. Using the basic procedures outlined below, the expression of CD86 on the cell surface and cell viability are determined by flow cytometry, and the data is used to determine cell viability and CD86 stimulation index (S.I.). Then the CV70 (concentration showing 30% cytotoxicity) and EC150 (concentration at which the CD86 S.I. is 150% of control) are then calculated, if applicable.

Accurate & reliable data

Fast turnaround times

Unsurpassed expertise

Collaborative approach

Calculations for CD86 S.I. are performed as follows:

$$\text{S.I.} = \frac{\%\ \text{of CD86* treated cells - \% of IgG1* treated cells}}{\%\ \text{of CD86+ control cells - \% of IgG1+ control cells}} \times 100$$

Based on the viability and CD86 S.I., the test articles can be classified according to Figure 1. It is important to note that the sensitization hazard assessment of a test article cannot be determined using the U-SENS assay alone. The results must be taken in context of other in vitro assays (OECD 442C and OECD 442D).

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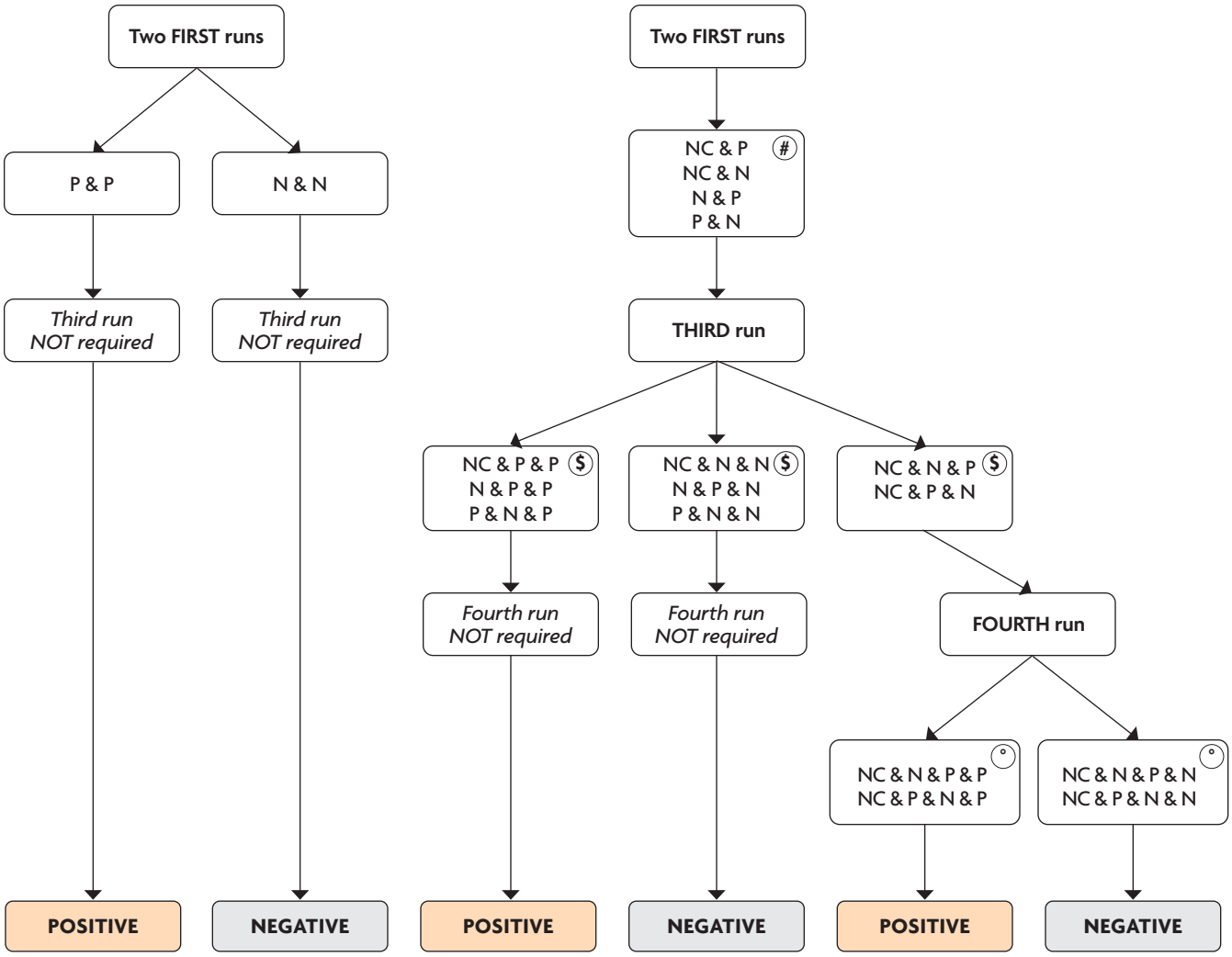
Figure 1. U-SENS prediction model for determination of sensitization potential.*



N: Run with no CD86 positive or interference observed;

P: Run with CD86 positive and/or interference(s) observed;

NC: Not Conclusive. First run with No Conclusion when CD86 is positive at the highest non-cytotoxic concentration only;

#: A Not Conclusive (NC) individual conclusion attributed only to the first run conducts automatically to the need of a third run to reach a majority of Positive (P) or Negative (N) conclusions in at least 2 of 3 independent runs.

$: The boxes show the relevant combinations of results from the three runs on the basis of the results obtained in the first two runs shown in the box above.

°: The boxes show the relevant combinations of results from the four runs on the basis of the results obtained in the first three runs shown in the box above.

***** A U-SENS prediction should be considered in the framework of a DA (defined approach) or an IATA (Integrated Approach to Testing and Assessment) and in accordance with the provisions of OECD 442E.

Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	U937 cell line
Replicates	3
Solvent of Choice	Culture media (preferred) or dimethyl sulfoxide (DMSO)
Test Article Formulation	200, 100, 50, 20, 10, and 1 µg/mL
Solvent Controls	Culture media (preferred) or dimethyl sulfoxide (DMSO)
Positive Control	Picrylsulfonic Acid (2,4,6-Trinitro-benzene-sulfonic acid; TNBS; CAS 2508-19-2)
Negative Control	Lactic acid (CASRN 50-21-5)
Exposure Time	45 ± 3 hours
Viability and CD86 Expression	Flow Cytometry
Time to Complete	4-6 weeks from test article receipt
Regulatory	Non-GLP or GLP
Deliverables	Full Report, Sensitization potential (positive or negative)

Key References

UN (2013), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth Revised Edition, UN New York and Geneva.

OECD (2014), The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence, Series on Testing and Assessment No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

FDA (2023) Guidance Document: Nonclinical Evaluation of the Immunotoxic Potential of Pharmaceuticals, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, FDA-2019-D-5607.

OECD (2024), Test No. 442C: In chemico skin sensitisation assays addressing the Adverse Outcome Pathway Key Event on Covalent Binding to Proteins, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264229709-en.

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OECD (2024), Test No. 442E: In Vitro Skin Sensitisation Assays Addressing the Adverse Outcome Pathway Key Event on Activation of Dendritic Cells, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264359-en.