FHAIVE
Finnish Hub for Development and Validation of Integrated Approaches

Jack Morikka 29.03.23

Alternative Methods in Toxicity Studies

fimea

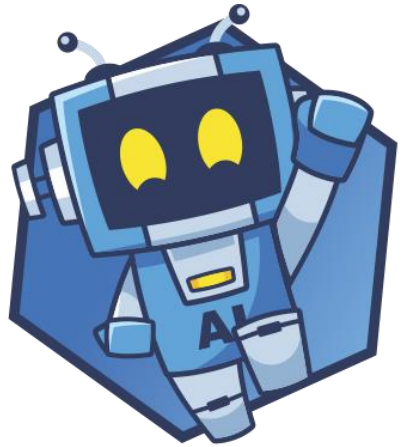
FHAIVE (est. 2021)

- FHAIVE is a research hub in the Faculty of Medicine and Health Technology, Tampere University.
- FHAIVE is the GLP national reference laboratory of Finland for validation of alternative methods (ECVAM)
- FHAIVE offers GLP IATA, OECD tests, and validation services
- Annual budget ~2M € (competitive funding)
- In FHAIVE, IATA are developed by integrating advanced *in vitro* models with toxicogenomics and AI-enabled advanced data modelling

Jack Morikka

- M.Sc. Molecular & Cellular Biology (2012), Glasgow University
- Ph.D. 2020, Tampere University
- Postdoctoral researcher, University of Helsinki 2020-2022
- FHAIVE researcher & GLP Study Director since 2022
- Expertise in Metabolomics, in vitro assays, mechanistic toxicology


Reebok
BIOPSI
D'OH!



ARCHIMEDES

Tests under GLP at FHAIVE

Test/service	Principle
Acute cytotoxicity test	Acute toxicity testing using mouse BALB 3T3 or BJ fibroblasts, with Neutral Red Uptake Assay in compliance with OECD GD 129.
EpiDerm™ skin corrosion test	EpiDermTM human cell based three-dimensional differentiated keratinocyte cultures (EpiDermTM), for testing corrosion of chemicals in compliance with OECD TG 431.
EpiDerm™ skin irritation test	EpiDermTM human cell based three-dimensional differentiated keratinocyte cultures (EpiDermTM), for testing irritation of chemicals in compliance with OECD TG 439.
ISO 10993-5:2009 “Biological evaluation of medical devices: tests for in vitro cytotoxicity”	Cytotoxicity testing using human BJ fibroblasts and Neutral Red Uptake Assay in compliance with ISO 10993-5:2009

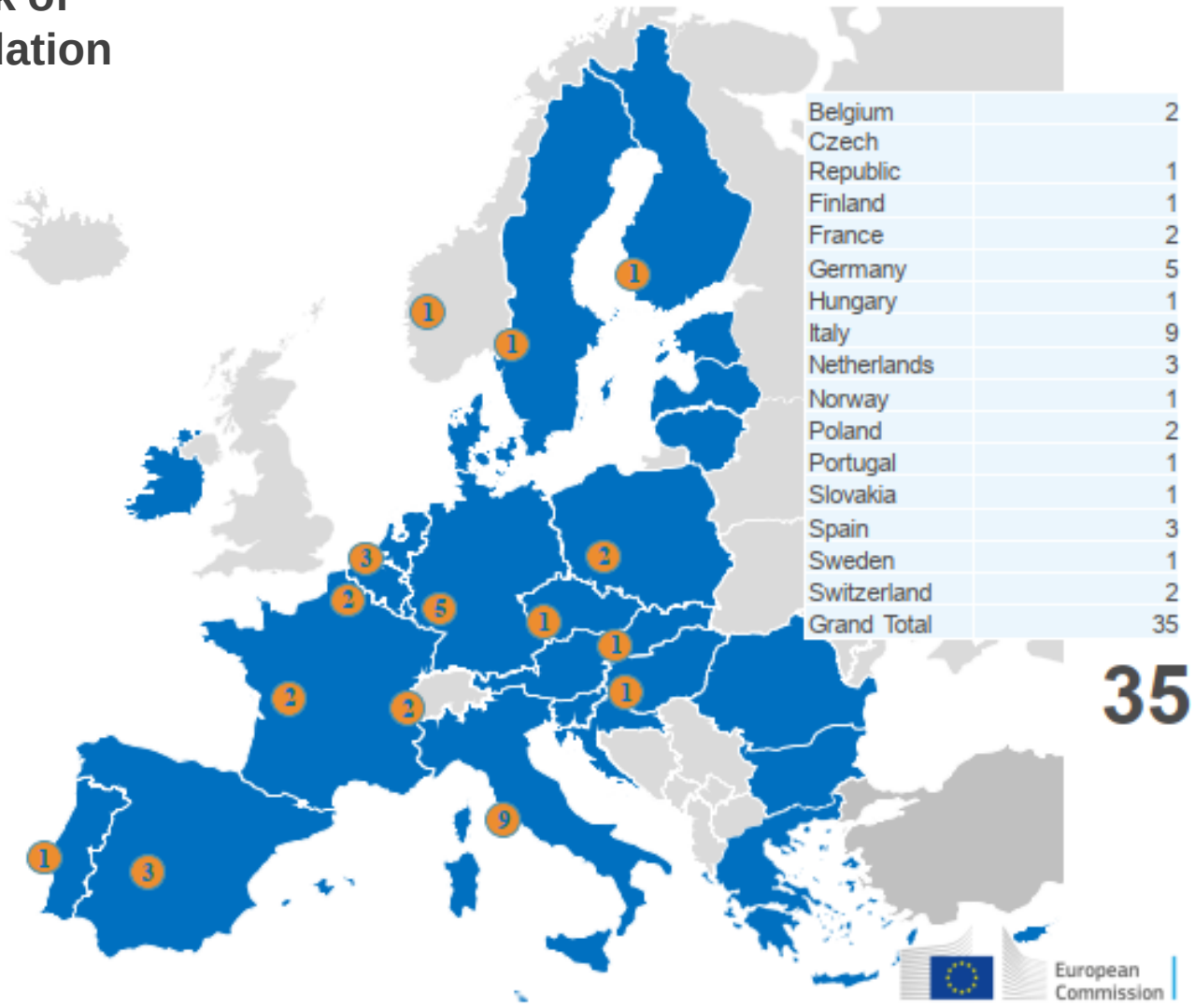
Note that these are all in vitro tests

EU-NETVAL
(European Union Network of Laboratories for the Validation of Alternative Methods)

European Commission

Joint Research Centre
JRC

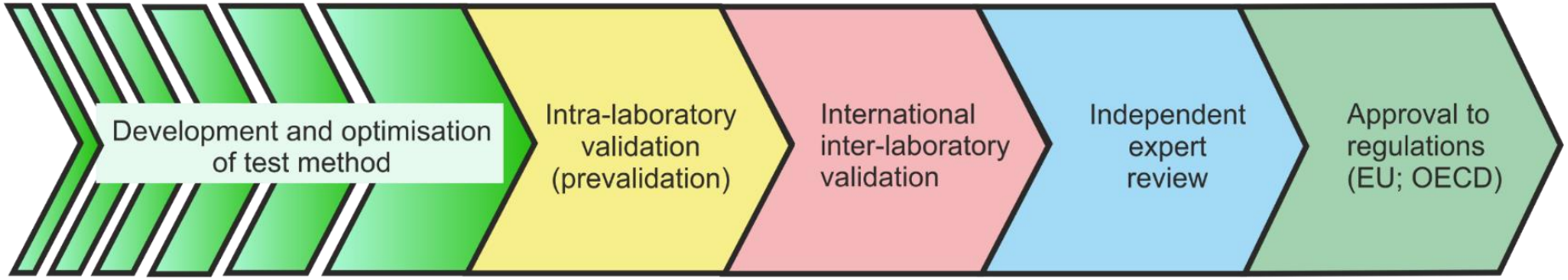
EURL
ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing



mmm.fi
MINISTRY OF AGRICULTURE AND FORESTRY

Ministry of Education and Culture

FHAIVE TEST VALIDATION



As an example, FHAIVE has recently validated, with ECVAM assessment, an internally developed in vitro vasculogenesis/angiogenesis (VA) assay, as a method for the detection of thyroid disruptors.

FIN3R Centre in 2023

Web page https://fin3r.fi/

Chair: Prof. Eero Lehtonen (TOKES)

Leading group

Coordinator: Prof. Dario Greco

Replacement director: Dr. Johanna Englund

Reduction director: Prof. Mikko Airavaara

Refinement director: Dr. Vootele Vöikar

Advisory group

Replacement	Reduction	Refinement	Coordinating
Jenni Hakkarainen	Satu Kuure	Johanna Åhlgren	Tommaso Serchi
Virve Sihvola	Reetta Hinttala	Brian Mphande	
Sarka Lehtonen	Francisco Lopez Picon	Rami Kuhanen	

Web page and social media responsible: Sara Sladakovic

LIMITATIONS OF TRADITIONAL TOXICOLOGY

- The majority of currently available methods only measure physiological endpoints (e.g. cell death) but give no insight on underlying mechanistic features, missing valuable information including potentially unseen toxic effects.

- Extrapolation of results from these current in-vitro tests, to a determination of results in in-vivo systems is very limited.

- Current approaches, particularly those using animal models, are expensive, time inefficient, and resource intensive.

- A lack of throughput for current test methods limits the number of substances that can be tested.

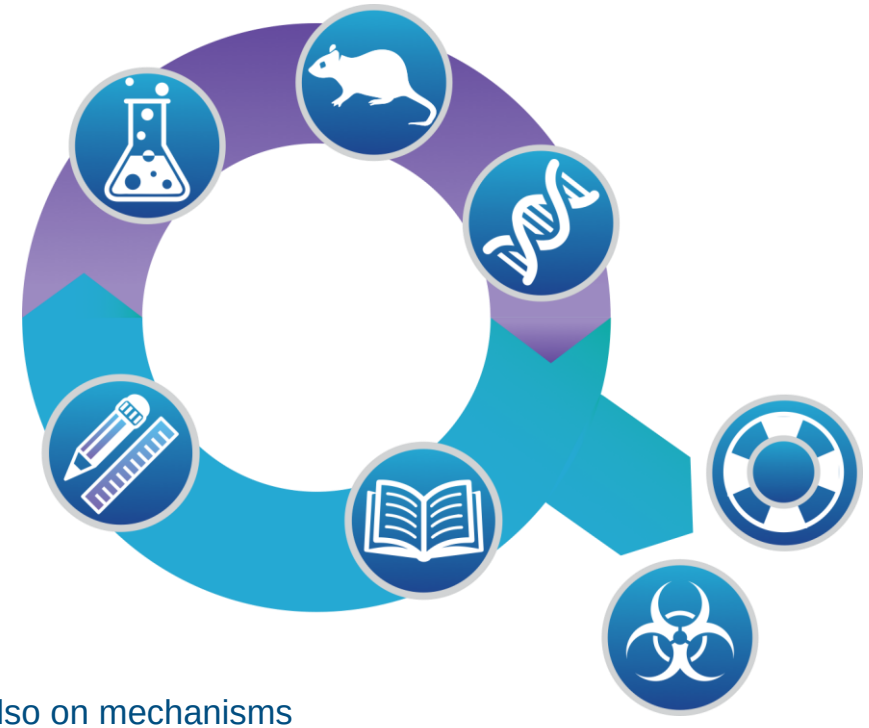
- **Current tests have no predictive capability**

Current paradigms in toxicology

Traditional toxicology

- One chemical at a time
- Focuses mainly on phenotypic effects
- Limited knowledge to design new compounds

Systems toxicology



- Focuses also on mechanisms
- Knowledge to design new compounds
- Lacks standardisation, not regulatory accepted

FHAIVE APPROACHES (WHERE THE REGULATORY FIELD IS HEADED).

New Approach Methods (NAM)

Quantitative In Vitro to In Vivo Extrapolation (QVIVE)

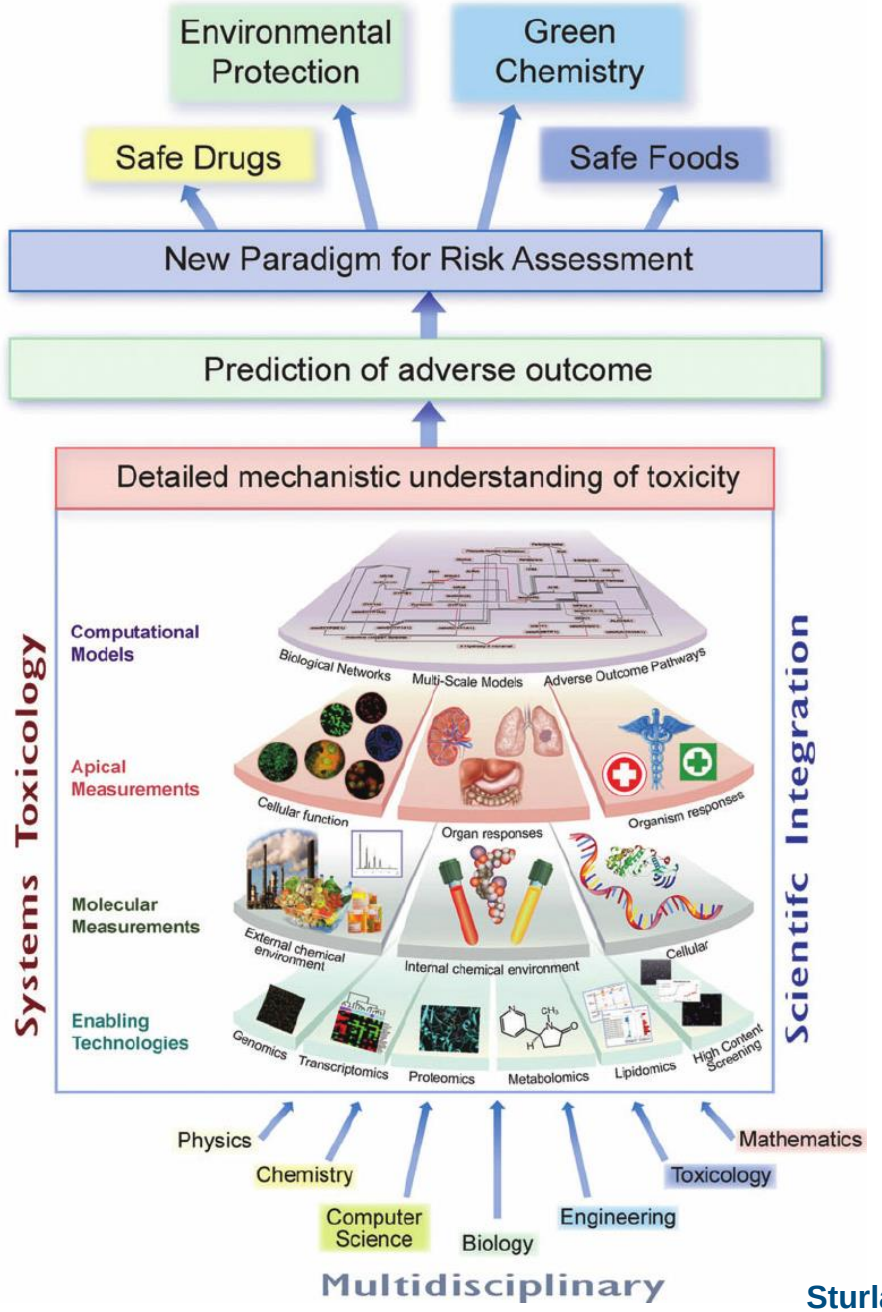
Integrated Approaches to Testing and Assessment (IATA)

Adverse Outcome Pathways (AOP)

SUBJECT	OVERVIEW	GLP
New Approach Methods (NAM)	Computational modeling and advanced in vitro models to avoid animal experimentation in fitting with the 3R principles. More informative than traditional animal toxicology.	All NAMs must be thoroughly tested for usefulness and reproducibility of method before being brought into use for hazard and risk assessment
Quantitative In Vitro to In Vivo Extrapolation (QVIVE)	Increased mechanistic understanding of toxic exposures allows quantitative modelling of how results from in-vitro models transfer to in-vivo models.	Current GLP tests give insufficient output and mechanistic understanding to extrapolate in-vitro results to in-vivo. This needs to change. OMICs technologies in particular will power GLP-QVIVE
Integrated Approaches to Testing and Assessment (IATA)	Chemical hazard characterization using integrated analysis of e.g. QSAR, read cross, in-vitro, toxicogenomics, to improve mechanistic understanding	Combined knowledge from a multitude of bioinformatic and in-vitro approaches can greatly expand the risk assessment of toxic substances in GLP
Adverse Outcome Pathways (AOP)	A systematic framework, characterizing mechanistic information from toxic exposures into formalised pathways with key events.	Can be used as a framework for e.g. IATA output and bring robustness of mechanistic data into a GLP format (reproducible data and understandable data)

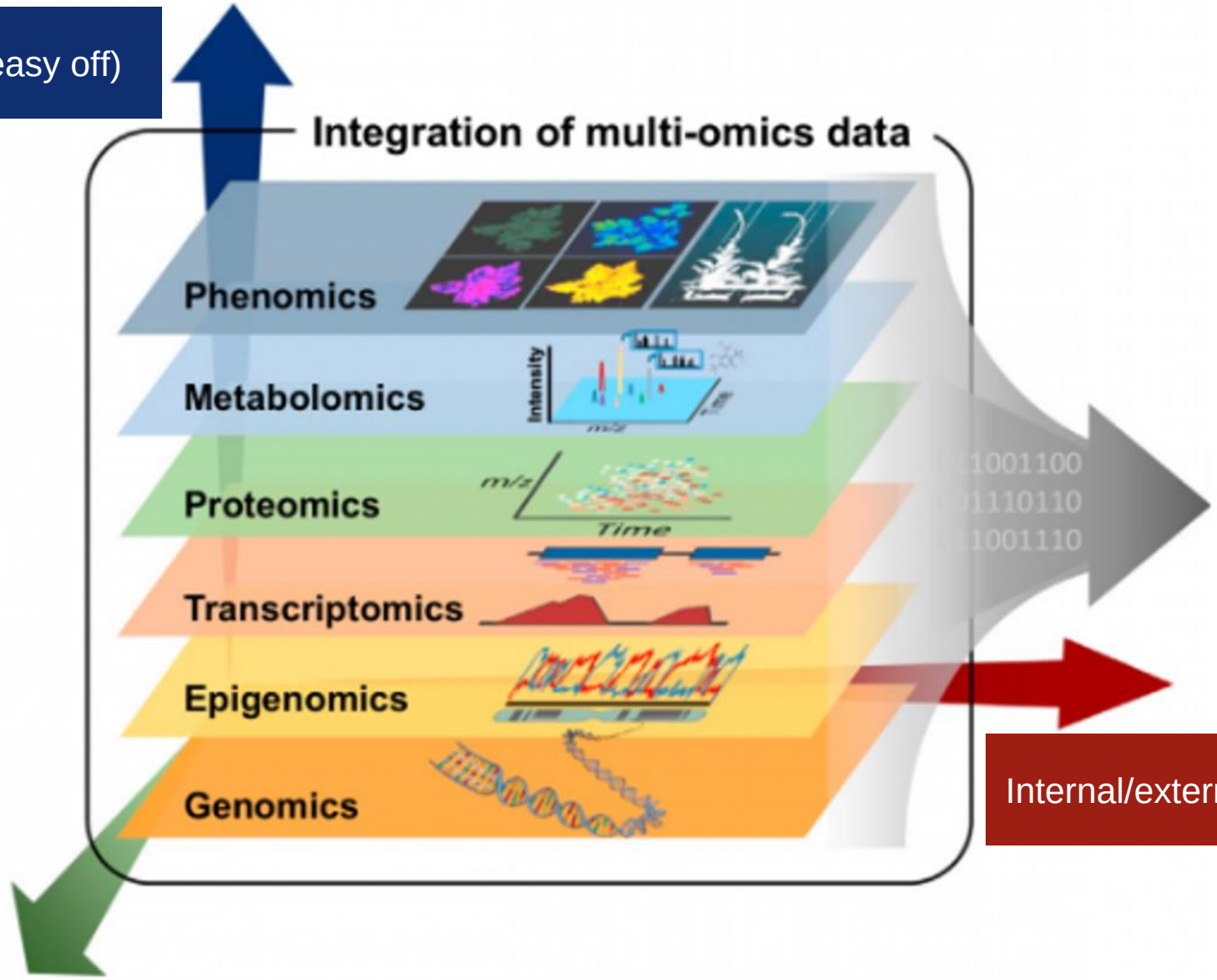
NAMs & IATA

- **Mechanistic models**
- **Integration of multiple NAMs to generate IATA**
- **Higher degree of output complexity (more sophisticated data analysis needed)**



Sturla *et al.* 2014

Instability (fast on - easy off)

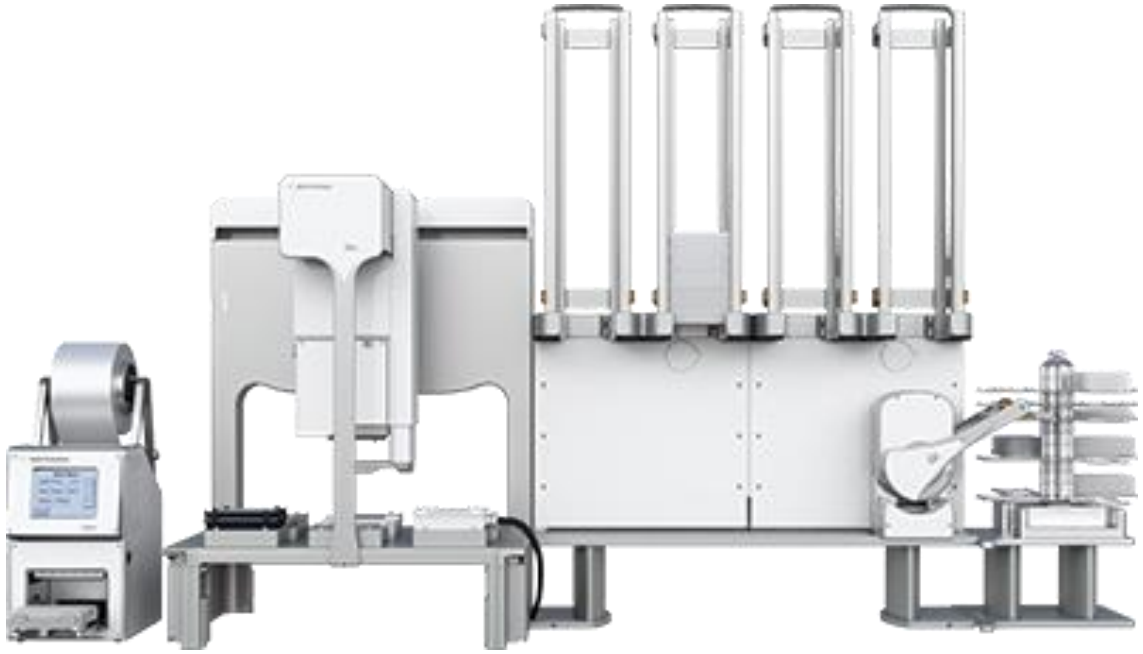


Internal/external stimuli

Multi-scale (cells, tissues, organs, etc)

Kim et al., DOI: 10.1016/j.molp.2016.04.017

Automated Pipetting Workstation for High Throughput

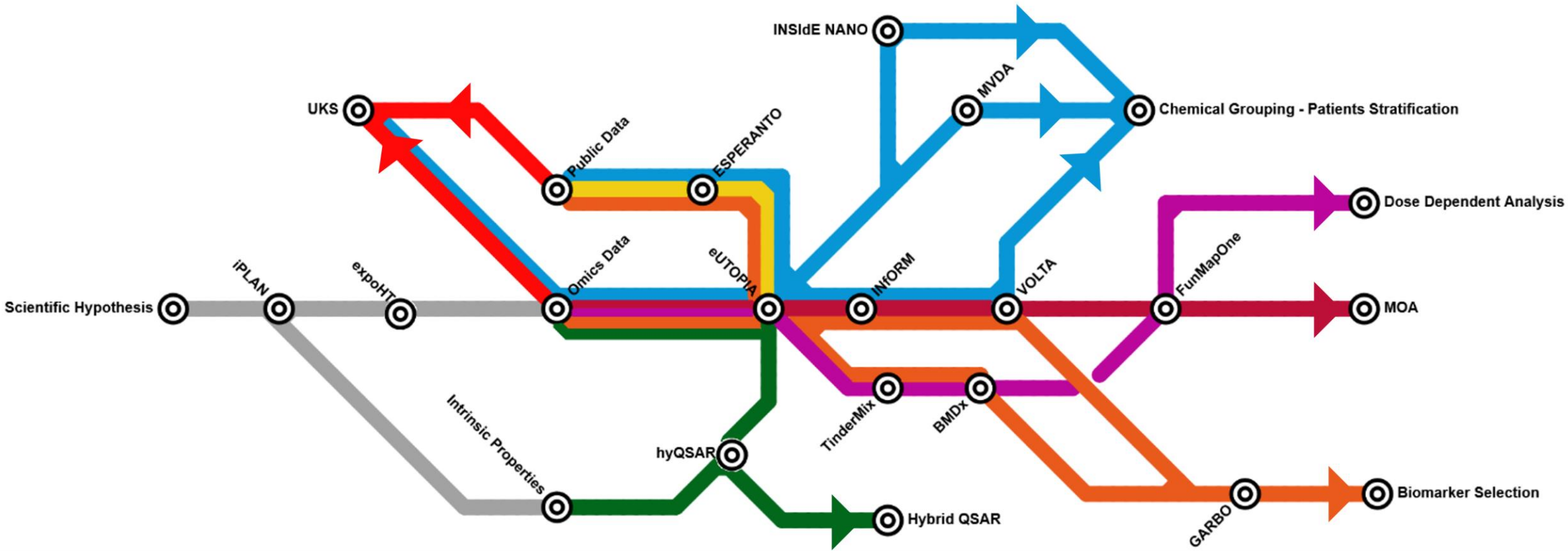


3D Cell/Gel Bioprinter

Tampere University

NEXTCAST

bypass to knowledge...



https://github.com/fhaive/nextcast

Serra *et al.* **Bioinformatics 2015**

Marwah *et al.* **BioInformatics 2018**

Marwah *et al.* **Source Code Biol Med 2019**

Serra *et al.* **Scientific Reports 2019**

Scala *et al.* **Bioinformatics 2019**

Fortino *et al.* **Bioinformatics 2020**

Serra *et al.* **GigaScience 2020**

Serra *et al.* **Bioinformatics 2020**

Serra *et al.* **Bioinformatics 2020**

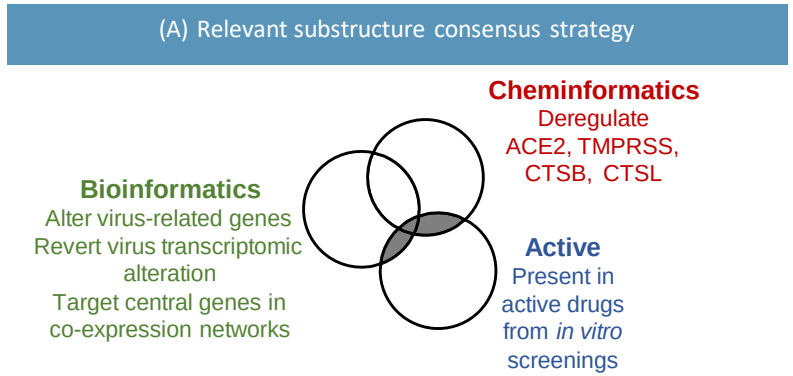
Scala *et al.* **Bioinformatics 2021**

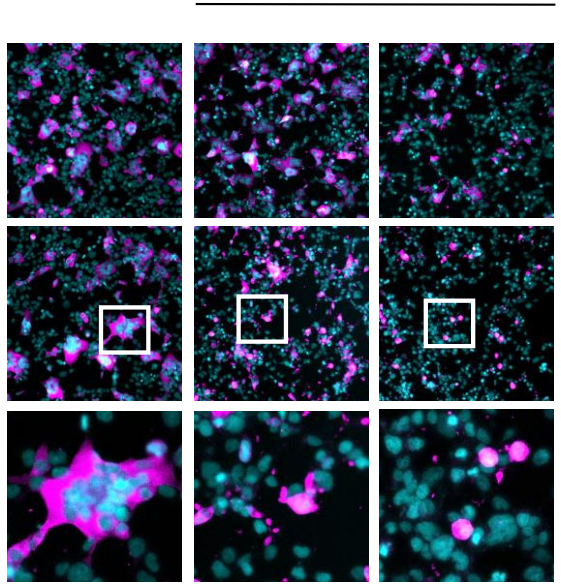
Pavel *et al.* **Bioinformatics J. 2021**

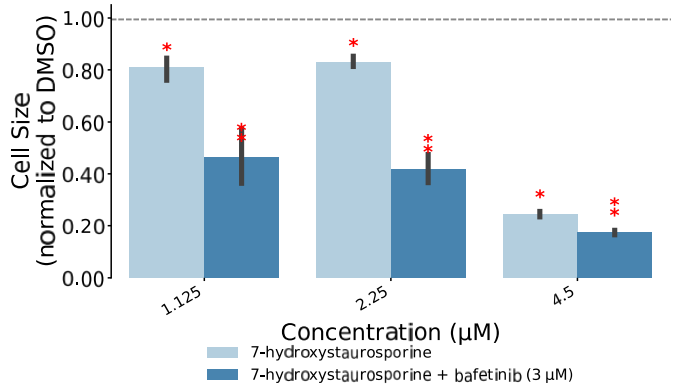
Pavel, del Giudice *et al.* **Briefings in Bioinformatics J. 2021**

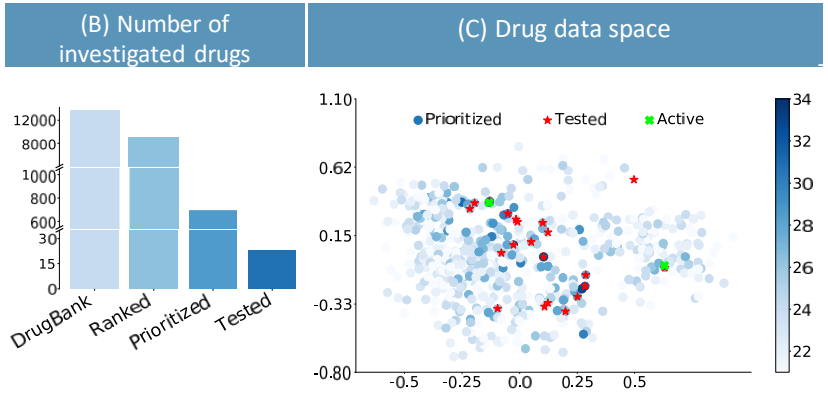
Serra *et al.* **Comp. Struct. Biotech. J. 2022**

Integrative modelling for COVID-19 drug discovery







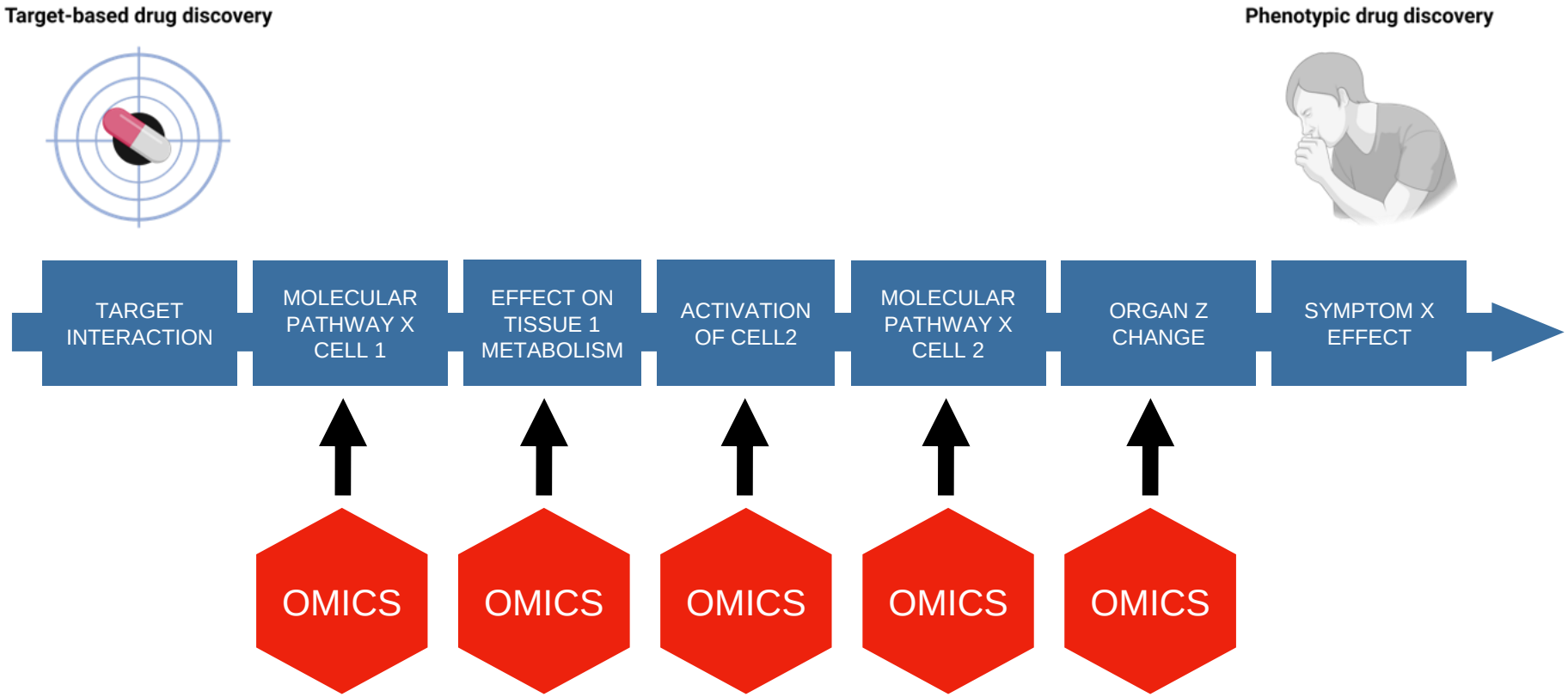


Serra & Fratello *et al.* **Brief. Bioinfo.** 2022

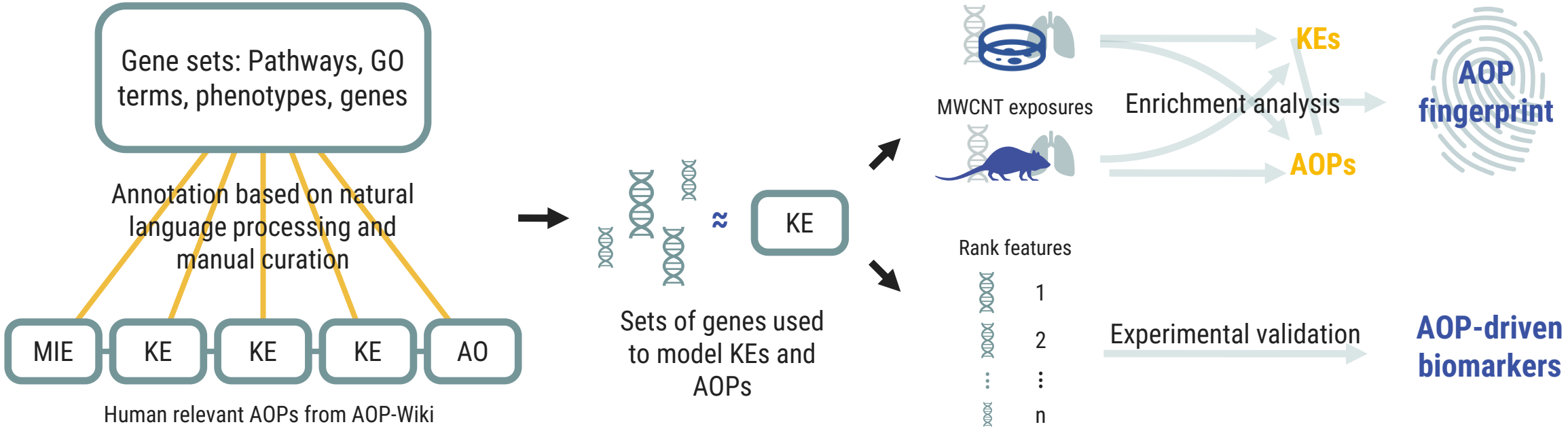
AOP/TOP & omics in drug development

ADVERSE OUTCOME PATHWAYS (AOP) / THERAPEUTIC OUTCOME PATHWAY (TOP)

- Multi-scale cause-effect models in biomedicine
- Chains of related key events
- OECD regulatory acceptance



AOP-based development of new integrated tests



Saarimäki *et al.* **Adv. Sci.** 2022

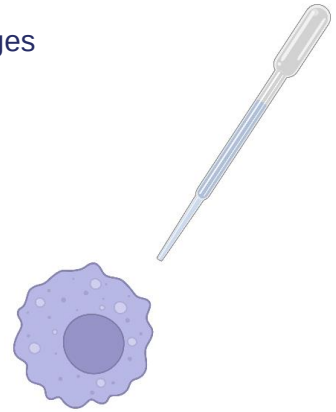
AOP-driven biomarkers

Gene prioritisation and experimental validation

Characteristics for transcriptional biomarkers based on the Bradford Hill criteria

Bradford Hill	Our characteristic	Method/Assessment
Consistency (reproducibility)	Reproducibility	Selection considers evidence from previous profibrotic exposures
Strength (effect size)	Amplitude	Significant alteration of the expression as compared to control
Experiment	Measurable	Transcriptional biomarkers measurable by qPCR; selected genes need to be expressed in the model
Biological gradient (dose-response relationship)	Dose-responsive	Benchmark-dose modelling to evaluate dose-response
Coherence	*In vitro* to *in vivo* extrapolation	Experimental evidence from *in vitro* and *in vivo*[1)]
Analogy	Predictive (of the outcome of interest)	Selection based on the KEs preceding the AO of interest
Specificity	Specificity	Gene ranking based on the specificity score
Plausibility	(Biological) plausibility	The AOP framework provides a plausible context; supporting evidence; selection of the organism
Temporality	Temporality	Transcriptional alteration follows the exposure; selection of the model organism[2)]
–	GLP-method	RT-qPCR
–	Influence	Centrality measures from human protein-protein interaction and gene regulatory networks

- Bleomycin (known pro-fibrotic exposure) on THP-1 macrophages
- RT-qPCR
- Dose-response analysis



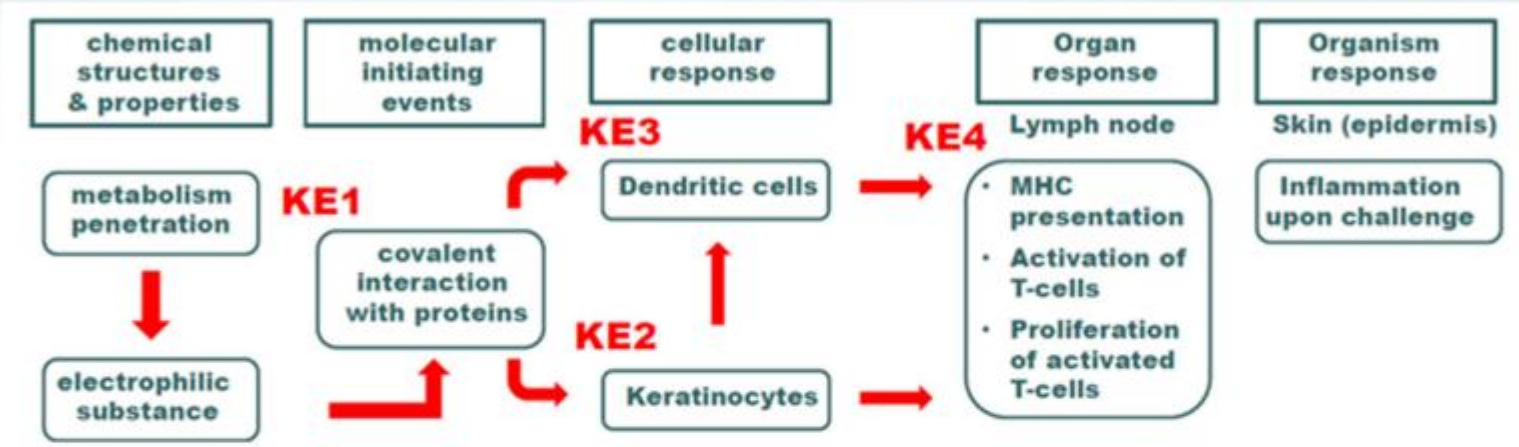
AOP-derived *in vitro* biomarkers for lung fibrosis

CXCL2 CCL7 TGFBI

IL8 MMP19

Saarimäki *et al.* **Adv. Sci.** 2022

Adverse Outcome Pathway and Predictive Testing



DPRA Direct Peptide Reactivity Assay
ADRA *Amino acid Derivative Reactivity Assay*

Are-Nrf2 Luciferase test methods: **Keratinosens™** **LuSens**

h-CLAT Human Cell line Activation Test
U-SENS™ U937 Cell line activation test
IL-8-Luc Interleukin-8 Reporter Gene Assay

OECD 442C
In chemico (KE1)

OECD 442D
In vitro (KE2)

OECD 442E
In vitro (KE3)

Human Safety
Ensuring Safe Products

https://www.cir-safety.org/sites/default/files/160th%20CIR%20EP%20Skin%20Sensitization%20NAM%20Upate%20Don%20Bjerke%20Final%20updated.pdf

GARD®skin.

OECD TG 442E:
in vitro skin sensitization

GARDskin is a robust *in vitro* test to identify potential chemical skin sensitizers with broad applicability and over 90% prediction accuracy. The test provides a binary prediction, classifying the test samples into either a skin sensitizers or non-sensitizer.

Approved by OECD for regulatory testing

As a new method included in OECD TG 442E for *in vitro* skin sensitization, GARDskin supports discrimination of skin sensitizers and non-sensitizers in accordance with the UN GHS.

Your stand-alone test for product development

With demonstrated high performance and broad applicability, GARDskin is appreciated across industries as a stand-alone product development *in vitro* tool for skin sensitization hazard assessment.

Based on your needs within *in vitro* skin sensitization, GARDskin Dose-Response is available as a test option for quantitative potency assessment.

Do you have "difficult-to-test" samples?

GARDskin works for a wide variety of test chemicals, with demonstrated applicability to evaluate "difficult-to-test" samples, including:

- Complex mixtures
- Indirectly acting haptens
- Lipophilic compounds
- Metal and metal salts
- Solid materials
- Surfactants

VISIT "DIFFICULT-TO-TEST" PAGE › GET A QUOTE

PUBLISHED JUNE 2022

How it works

GARDskin uses a human dendritic-like cell line, SenzaCell™, which mimics a critical part of the human immune system and is able to recognize allergens.

In each test case, the cells are exposed to the test sample after which genomic biomarker signature is measured. The gene expression pattern of the exposed cells is then compared to existing patterns induced by well-known chemicals and analysed by pattern recognition and machine-learning technology. As a result, the test sample is classified as a sensitizer or non-sensitizer.

All the GARD assays are based on the same technology platform. Read more about the GARD technology platform and assay development principles on the Science page of our website.

https://www.oecd-ilibrary.org/docserver/9789264264359-en.pdf?expires=1677075723&id=id&accname=ocid177564&checksum=335635679446803CC4D3BD8F23C598F0

The future of GLP at FHAIVE

FHAIVE is bringing the forefront of toxicology to Finland, ensuring robust efficacy and safety testing of chemicals and biologicals with New Approach Methods (NAMs).

FHAIVE uses cutting edge artificial intelligence and in-vitro cell models to predict adverse outcomes or potential therapeutic applications from chemicals and substances.

FHAIVE is working to develop and validate these new technologies in the scope of toxicology testing under GLP.

Thank you for your attention.

Tampere University