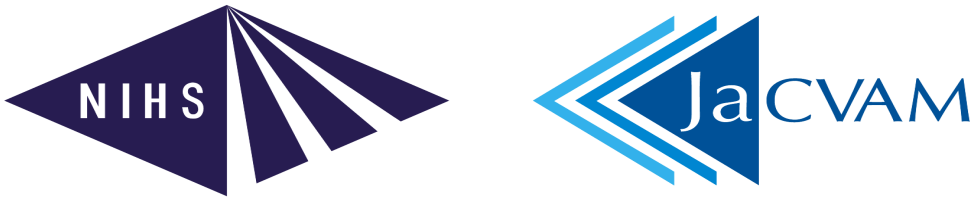
OpenTox Asia 2018,
May 24th, 2018, Tokyo, Japan

New trend on alternative to animal testing in Japan



Hajime Kojima

National Institute of Health Sciences (NIHS), Japan

JaCVAM: Japanese Center for the Validation of Alternative Methods

This Center was established at the National Institute of Health Sciences (NIHS) in Japan, 2005 by the Ministry of Health, Labour and Welfare (MHLW).

JaCVAM mission

- To promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan.
- To establish guidelines for new alternative experimental methods through international collaboration.

ICATM

ICATM is a **voluntary** international cooperation of national organizations: Canada, the European Union, Japan, South Korea, and the United States.

APPLY INTEGRATED APPROACHES TO TESTING AND ASSESSMENT

All of the work on alternative methods is undertaken at the OECD with the objective of contributing to more integrated approaches to testing and assessment. In practice, integrated approaches, which take into account the tools outlined above, are used in the OECD Existing Chemicals Programme which generates internationally agreed initial hazard assessments of chemicals.

This practical application of integrated approaches improves their regulatory acceptance and facilitates their implementation into national and regional chemical assessment schemes in OECD member countries.

AVOID DUPLICATION OF TESTING

The OECD **Mutual Acceptance of Data** (MAD) framework has had a major impact on testing practices. MAD guarantees that data generated in the testing of chemicals in an OECD member country, or adhering non-member country, in accordance with OECD Test Guidelines and OECD Principles of Good Laboratory Practice shall be accepted in other member or adhering countries for purposes of chemical assessment and other uses relating to the protection of man and the environment. This proactive framework saves thousands of animals every year and its impact increases as non-OECD economies join the MAD system.

Furthermore, the OECD has developed the **Global Portal to Information on Chemical Substances** (eChemPortal). eChemPortal offers free public access to information on properties of chemicals through a simultaneous search of multiple databases, thereby improving the access to existing test results and reducing the risk of unnecessary testing.

WHERE CAN I FIND OECD TOOLS RELATED TO CHEMICAL SAFETY AND ANIMAL WELFARE ?

(Q)SARs, Grouping of Chemicals and the (Q)SAR Application Toolbox

www.oecd.org/env/existingchemicals/qsar

Test Guidelines, *in vitro* test methods, molecular screening and toxicogenomics

www.oecd.org/env/testguidelines

Integrated Approaches to Testing and Assessment

www.oecd.org/env/existingchemicals

Mutual Acceptance of Data

www.oecd.org/env/glp

Global Portal to Information on Chemical Substances

www.oecd.org/ehs/eChemPortal

For more information contact
the OECD Secretariat at
ehscont@oecd.org

Chemical Safety and Animal Welfare

Progress made at the OECD

www.oecd.org/env/ehs

Non-animal alternative test methods in the OECD TG(2018)

Class	Test methods
Corrosion	*In vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER) :TG430
	In vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) test method :TG431
	CORROSITEX Skin Corrosivity Test :TG435
Skin irritation	*In vitro* Reconstructed Human Epidermis (RhE) Test methods, EpiDerm, EPISKIN, SkinEthic, LabCyte EPI-Model: TG439
Phototoxicity	3T3 NRU Phototoxicity Test :TG432
Eye irritation	Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage:TG437
	Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage:TG438
	Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants：TG460
	Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage：TG491
	Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage：TG492
Skin sensitisation	*In Chemico* Skin Sensitisation, Direct Peptide Reactivity Assay (DPRA) :TG442C
	In Vitro Skin Sensitisation, ARE-Nrf2 Luciferase Test Method :TG442D
	In Vitro Skin Sensitisation, Human Cell Line Activation Test : TG442E
Endocrine disrupter screening	Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists : TG455
	H295R Steroidogenesis Assay :TG456
	Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals: TG458
	Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity ：TG493
Genotoxicity	Bacterial Reverse Mutation Test : TG471
	In vitro Mammalian Chromosome Aberration Test : TG473
	In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes : TG476
	In vitro Micronucleus Test : TG487
	In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene ：TG490
Skin absorption	Skin Absorption: *In vitro* Method :TG428

OECD TG and GD developed and validated in Japan

- ✓ Skin sensitization assay, LLNA：DA, TG 442A (2010)
- ✓ Skin sensitization assay, LLNA：BrdU-ELISA , TG 442B (2010)
- ✓ *In vivo* comet assay TG 489 (2014)

- ✓ Skin irritation assay with LabCyte EPI-MODEL 24, TG 439 (2013)
- ✓ Performance-based Test Guideline for stably transfected transactivation *in vitro* assays to detect estrogen receptor agonists and antagonist, Revised TG 455 (2016)
- ✓ Short time exposure (STE) assay for eye irritation testing, TG491 (2015)
- ✓ Bhas 42 cell transformation assay (2016) Guidance document
- ✓ h-CLAT assay for skin sensitisation testing, TG442E (2016)
- ✓ Stable transfected transcriptional activation (STTA) assay for androgen disruptor screening (AR-Ecoscreen), TG458(2016)
- ✓ IL-8 Luc assay for skin sensitisation testing , TG442E (2017)
- ✓ LabCyte CORNEA-MODEL for eye irritation testing, TG492 (2018)

OECD/OCDE **442E**

Adopted:
9 October 2017

OECD TEST GUIDELINE FOR THE TESTING OF CHEMICALS BASED ON KEY EVENTS

In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome pathway for Skin Sensitisation

8. The test methods described in this Test Guideline cannot be used on their own, neither to sub-categorise skin sensitisers into subcategories 1A and 1B as defined by UN GHS (1), for authorities implementing these two optional subcategories, nor to predict potency for safety assessment decisions. However,

IATA (Integrated Approach on Testing and Assessment)

DA (Defined Approach)

Arch Toxicol. 2011 May;85(5):367-485. doi: 10.1007/s00204-011-0693-22011 May 1.

Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. Adler S, et al.,

In summary, the experts confirmed that it will take at least another 7-9 years for the replacement of the current in vivo animal tests used for the safety assessment of **cosmetic ingredients** for skin sensitisation.

For toxicokinetics, the timeframe was 5-7 years to develop the models still lacking to predict lung absorption and renal/biliary excretion, and even longer to integrate the methods to fully replace the animal toxicokinetic models.

For the systemic toxicological endpoints of repeated dose toxicity, carcinogenicity and reproductive toxicity, the time horizon for full replacement could not be estimated.

In vitro methods for reproductive toxicity

More than 30 different culture systems have been proposed

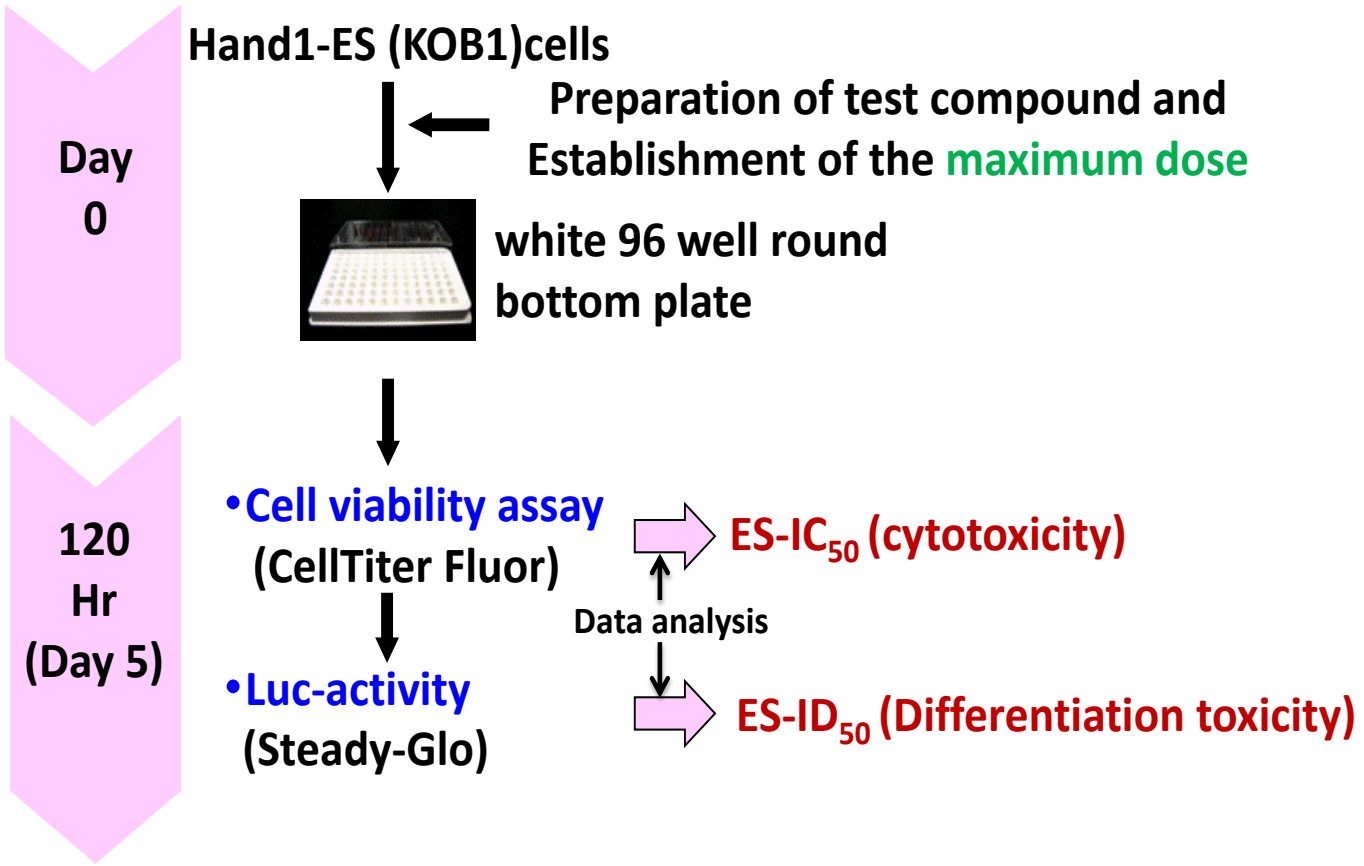
1) Tests on non-vertebrate species (*Hydra*, *Drosophila* etc.)
2) Tests on lower vertebrate embryo or embryonic aggregates (fish, birds etc.)
3) Tests on whole mammalian embryos
4) Tests on micromass cultures from mammalian embryos
5) Tests on embryonic stem cells (ES cells)
6) Tests on other mammalian cell lines (neuroblastoma cells, teratocarcinoma cells etc.)

(Food Chem. Toxicol.,2002 ,40,193)

No tests gain regulatory acceptance and use.

The research on alternative methods for detection of embryotoxicity is very challenging!

Hand1 gene related pathways



Hand1-Luc EST is an easy and inexpensive protocol

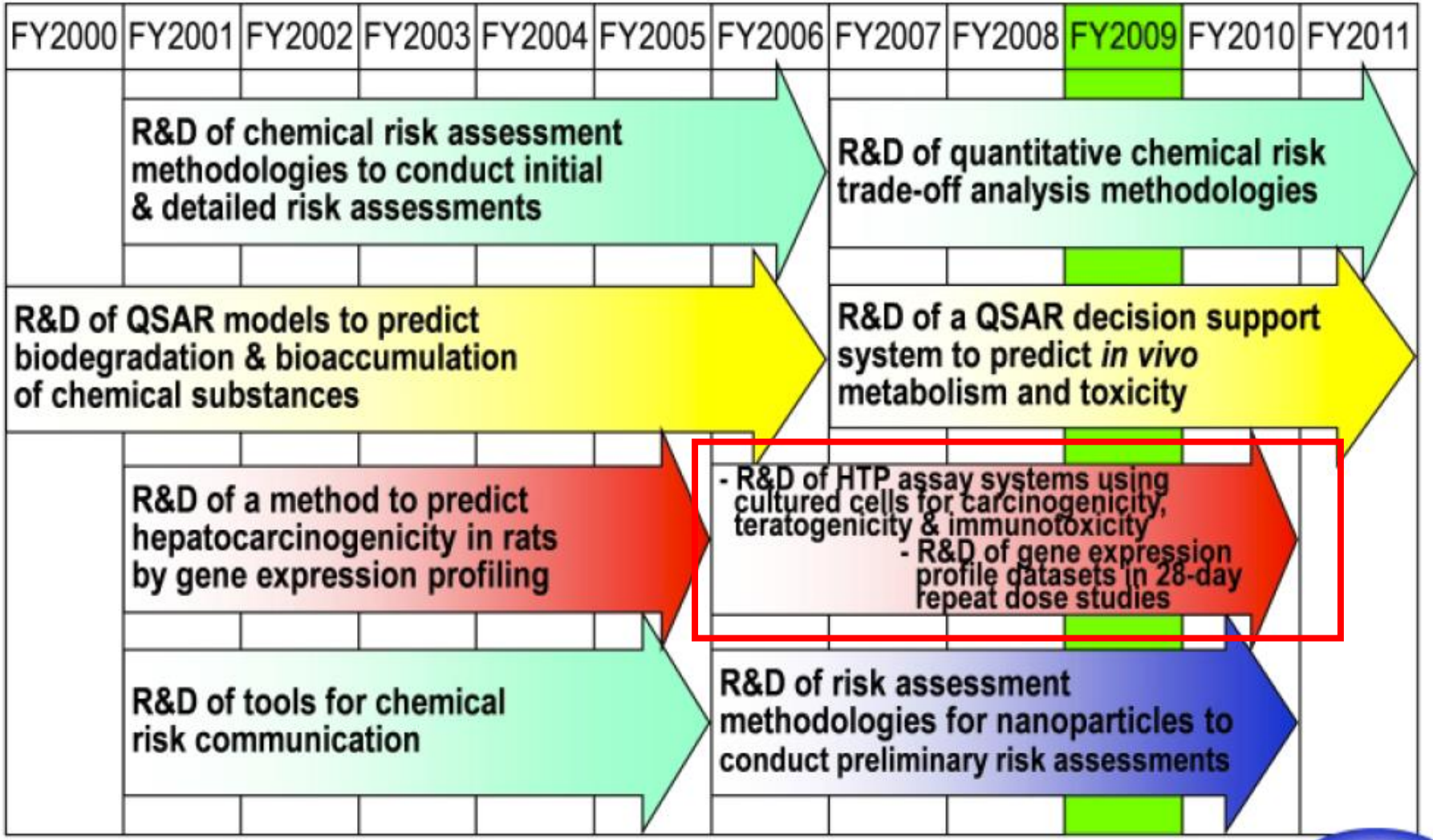
Test Guidelines Programme

DRAFT UPDATED WORK PLAN OF THE TEST GUIDELINES PROGRAMME

30th Meeting of the Working Group of the National Coordinators of the Test Guidelines Programme

Project 4.123: Review and feasibility of an Embryonic Stem Cell Test: In vitro assay detecting disruption to differentiation of rodent embryonic stem cells into cardiomyocytes using the Hand1 gene	
Lead: Inclusion in work plan: Project status and milestones:	Japan 2017
• 1st step: Detailed Review Paper of available methods and evaluation of utility and application; • 2nd step: feasibility study of the development of a Test Guideline (timelines are not provided yet).	
Subsidiary body of the JM	WNT
Expert group	

Strategic R&D of Chemical Risk Analysis Technologies by NEDO



In Vitro Alternatives

R&D of HTP assay systems using cultured cells for carcinogenicity, teratogenicity and immunotoxicity (1)

NEDO

HTP Assay for Carcinogenicity by Hatano Research Institute

Bhas 42 cells

- Cell transformation assay using 6-well plates → Cell transformation assay using 96-well plates → Robotic system
- Selection of marker genes → Multicolour luminescent reporter cell lines

HTP Assay for Teratogenicity by Sumitomo Chemical Co., Ltd.

Mouse embryonic stem cells → Differentiation into myocardial cells / neural cells → Selection of marker genes → Multicolour luminescent reporter cell lines

Confirmation

Rat whole embryo culture with metabolic activation (S9-mix addition) by Kamakura Woman's University

HTP Assay for Immunotoxicity by Tohoku University

Human immune cells Keratinocytes, Dendritic cells and T-cells → Selection of marker genes → Multicolour luminescent reporter cell lines

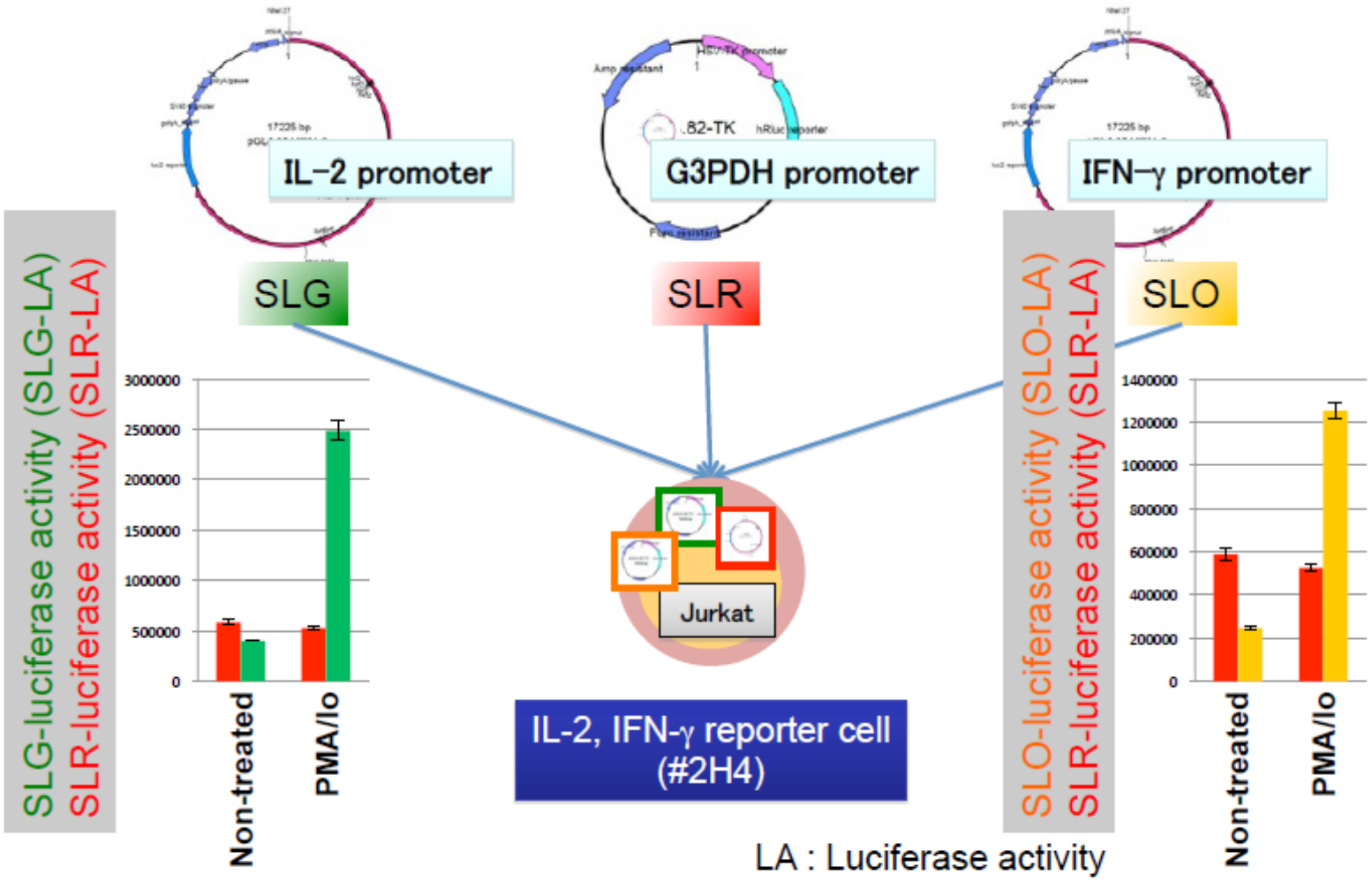
◆Formation of R&Ds

Technological support

1) Multicolour luminescent reporter cell method
by the National Institute of Advanced Industrial Science & Technology and Toyobo Co., Ltd.
enables to monitor the expressions of two or three marker genes in each cell simultaneously, promptly, and sequentially

2) Human artificial chromosome (HAC) vector method
by Tottori University
enables to deliver large size DNA segments and to evade transgene silencing caused by chromosomal position effect

IL-2, IFN-γ reporter cell (#2H4)



Aiba, S., et al, Tohoku Univ.

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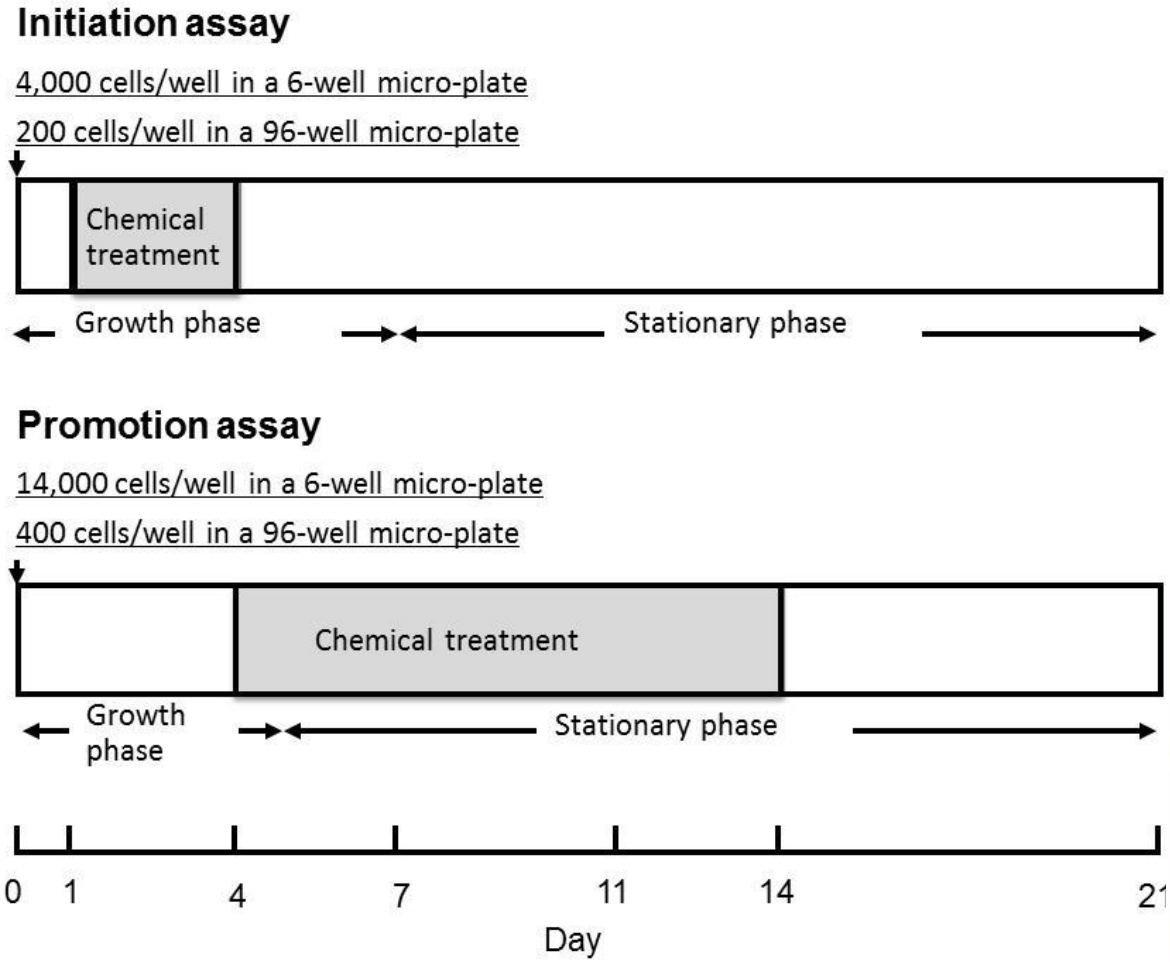
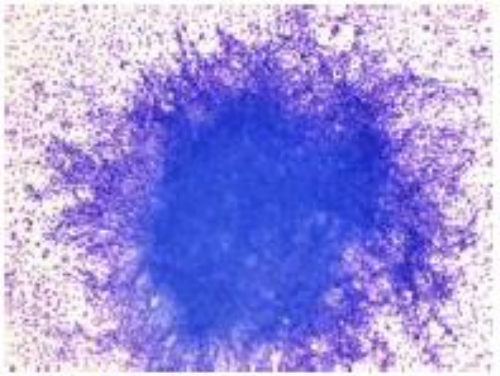
Illustration of the Bhas 42 cell transformation assaymethodology.





B+ S+ M++ R+ I+

The cells comprising the periphery of the focus are less densely packed and their spindle-shape and random orientation, although apparent, are less striking. The interlaced cells at the edge of the focus invade the surrounding monolayer.

A Project Focused on Developing Key Evaluation Technology Aiming at Industrialization of Regenerative Medicine: Development of Platform Technology for Drug Discovery through Application of Regenerative Medicine

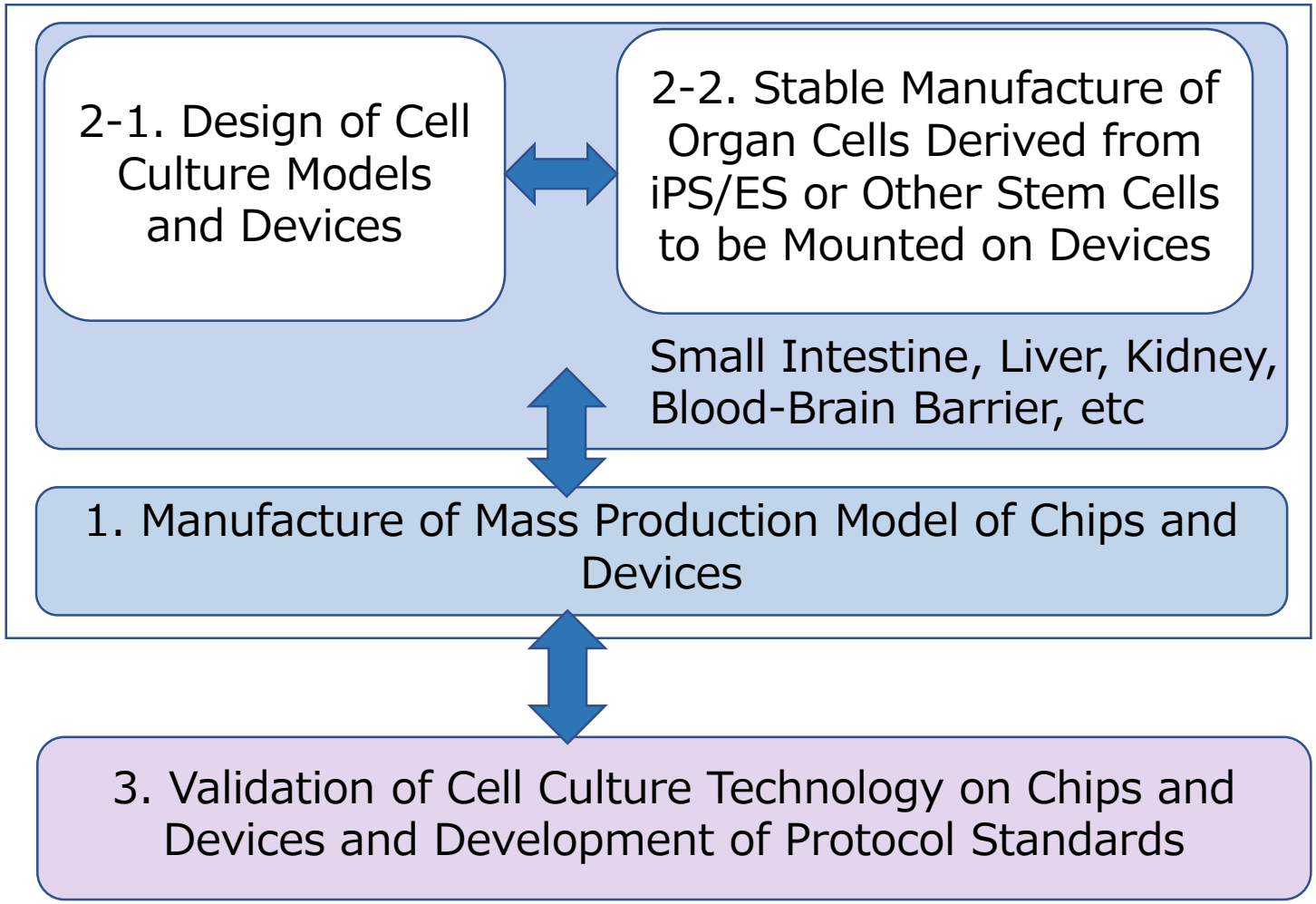
Background

AMED has been promoting the support tools for drug discovery using the induced pluripotent stem (iPS) cell-based technology to improve the efficiency of new drug discovery in “Japan Regenerative Medicine Project”.

This project aims to develop an organ(s)-on-a-chip system by mounting organ cells derived from iPS or other stem cells on chips and devices that can be applied for safety and pharmacokinetic evaluations in the process of drug discovery.

This project is implemented by collaborative efforts of designated project groups developing different parts of the organ(s)-on-a-chip system, which mainly comprises organ cell culture on chips and devices. Group 2-2 manufactures particular organ cells, which are derived from iPS or other stem cells, applicable to organ cell culture models and devices developed in Group 2-1. Group 1 develops a mass production model of chips and devices based on Group 2-1. Organ cell-culture technologies on chips and devices developed are then validated in Group 3, which develops protocol standards of the system.

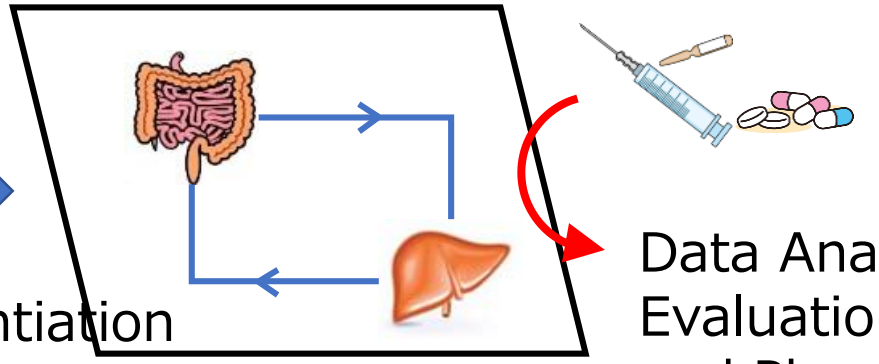
Development of Platform Technology for Drug Discovery through Application of Regenerative Medicine



Development of Platform Technology for Drug Discovery through Application of Regenerative Medicine

Candidates for New Drug

iPS cells or Other Stem Cells



Differentiation

Data Analysis
Evaluation of Safety and Pharmacokinetics

Chips and Other Devices with Mounted Organ Cells of the Small Intestine, Liver, Kidney, or Blood-Brain Barrier

METI consignment project

“Development of AI based next generation safety prediction system using related Big data”

AI-based
Substances
Hazardous
Integrated
Prediction
System project

Evaluation technology & Development project for Energy-saving electronic device materials
(Development of high-speed and efficient safety assessment technology making social implementation of functional materials)

--- Development of next generation integrated chemical safety prediction system by using A.I. and Chemical Toxicity related Big data

Ministry of Economy, Trade and Industry,
Manufacturing Industry Bureau,　Chemical Management policy Division.

Background

- Strengthen competitiveness of Japanese material/chemical industry
- Changes in the global business competitive situation
- Trend of reducing, replacement and refinement animal testing (3R)

Development policy and features of AI-SHIPS

- Unlike existing QSAR approach and other related prediction systems concept, we aim to develop the system that can predict chemical safety **based on mode of action i.e. AOP basis** and information of structural characteristics, physical properties of chemical substances.
- Conventionally, **ADME and PBPK** prediction methods that were not used in the such as prediction system can be drastically introduced, and to develop higher prediction accuracy system.
- By building a platform that unifies the DB of the existing chemical safety research and knowledge, we aim to develop the system **based on state-of-the-art artificial intelligence technology such as deep learning**
- For the elucidation of complicated and/or unique toxicity expression mechanism involving signal transduction mechanism etc., we will improve the system accuracy by reflecting the information that were obtained from comparison analysis of the data from **"Toxico-genomix"** approach with related toxicological end points.

Basic strategy for Develop a predictable system of the 28-day Repeated Dose Toxicity Test

1st STEP : To develop the prediction system of **Hepatotoxicity** (cytotoxicity, lipid abnormality, cholangiopathy and hypertrophy etc.) .
Hazardous and risk (NOEL)

2nd STEP : To develop **Hemato and Renal toxicity**.

- Industrial chemicals are not originally pursuing physiologically activity.
- Chemical structure is diverse e.g. the aliphatic chain to hetero ring, metal complex etc.
- Deepening the serious ecological health effects due to long-term exposure in trace amounts like PCB/TCDD, Organic Hg. Etc.

Future prospects

- Significant economical saving and 3Rs may be expected in the cost of Industrial chemical safety assessment which accounts for 10 years and 20% of R & D expenditure on chemical substances.
- Further, the term required for the toxicity test is set to substantially zero(5 min.?), and the development term can be greatly shortened.
- To promote the development of functional chemical substances by improving the efficiency of safety assessment in this way, and improve development capability and proposal capability of our functional materials and products using them.

Conclusion

For the systemic toxicological endpoints of repeated dose toxicity, immunotoxicity and reproductive toxicity, new test methods are expected to be developed in the future worldwide. I believe Japan will make a significant contribution to these developments in the Japanese projects.

Acknowledgement

- Delegate of AMED organ-on-chips project
- Delegate of AI-SHIPS project
- Delegate of NEDO project
- Delegate of JaCVAM steering committee

Office : New Testing Method Assessment, Division of Pharmacology,
National Biological Safety Research Center (NBSRC),
National Institute of Health Sciences (NIHS)


About JaCVAM


Update on JaCVAM


Academic activities


Submission of Alternative Methods to JaCVAM


International Cooperation

Thank you for attention

Policy and Mission: JaCVAM's policy and mission is to promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan and establish guidelines for new alternative experimental methods through international collaboration.

the 3Rs in animal experiments---Reduction (of animal use)
Refinement (to lessen pain or distress and to enhance animal well-being)
Replacement (of an animal test with one that uses non-animal systems or phylo-genetically lower species)
(OECD GD34)

News

- 【NEW】news texts dummy texts news texts dummy texts news texts dummy texts(2009.7.16)
- news texts dummy texts news texts (2009.7.3)
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