THE FUTURE OF IN VITRO SCREENING IN THE DEVELOPMENT OF NEW DRUGS

Hajime Kojima,
JaCVAM, NIHS, Japan

3Rs of animal use
(Russel and Burch 1959)

- 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)

Cosmetics Industry and the 7th Amendment of the EU Cosmetics Directive

- **EU: 2.000 companies, 60 billion € turnover**
- **EU: 5.000 new products per year, 25% turnover with products released within last 6 months**
- **Marketing ban since 2003 for testing finished products in animals or not using ECVAM-validated methods**
- **Phasing out testing in animals and stepwise marketing ban in 2009 and 2013**

7th Amendment of the EU Cosmetics Directive 76/768/EEC 2003

Ban of testing in animals

immediately ➔ since 2003

Testing of - finished products

Testing for - phototoxic potential
- skin penetration
- skin corrosion

Intensive research will be required to reach validation and regulatory acceptance of in vitro test for the following endpoints

31. Dec. 2009 - eye irritation
- skin irritation

31. Dec. 2013 - skin sensitization ?
- embryotoxicity ?
- repeat dose assay?

The Transatlantic Divide

Top-down development of new toxicological tools

Tox-21c

Johns Hopkins University
Center for Alternatives to Animal Testing

3Rs

Bottom-up support to alternative methods and legislative pressure

Regulatory Toxicology and Pharmacology 32, 56–67 (2000)
doi:10.1006/rtph.2000.1399, available online at http://www.idealibrary.com on IDEAL®

Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals

Harry Olson,[1] Graham Betton,[2] Denise Robinson,[3] Karluss Thomas,[3] Alastair Monro,[1] Gerald Kolaja,[4] Patrick Lilly,[5] James Sanders,[6] Glenn Sipes,[7] William Bracken,[8] Michael Dorato,[9] Koen Van Deun,[10] Peter Smith,[11] Bruce Berger,[12] and Allen Heller[13]

[1]*Pfizer Inc., Groton, Connecticut;* [2]*AstraZeneca Pharmaceuticals, Macclesfield, England;* [3]*ILSI-HESI, Washington, DC, 20036;* [4]*Pharmacia & Up John, Kalamazoo, Michigan;* [5]*Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut;* [6]*Rhone-Poulenc Rorer, Collegeville, Pennsylvania;* [7]*University of Arizona, Tucson, Arizona;* [8]*Abbott Laboratories, Abbott Park, Illinois;* [9]*Eli Lilly and Co., Greenfield, Indiana;* [10]*Janssen Research Foundation, Beerse, Belgium;* [11]*Monsanto-Searle Laboratories, Skokie, Illinois;* [12]*Sanofi-Synthelabo, Inc., Malvern, Pennsylvania; and* [13]*Bayer Corporation, West Haven, Connecticut*

Received January 22, 2000

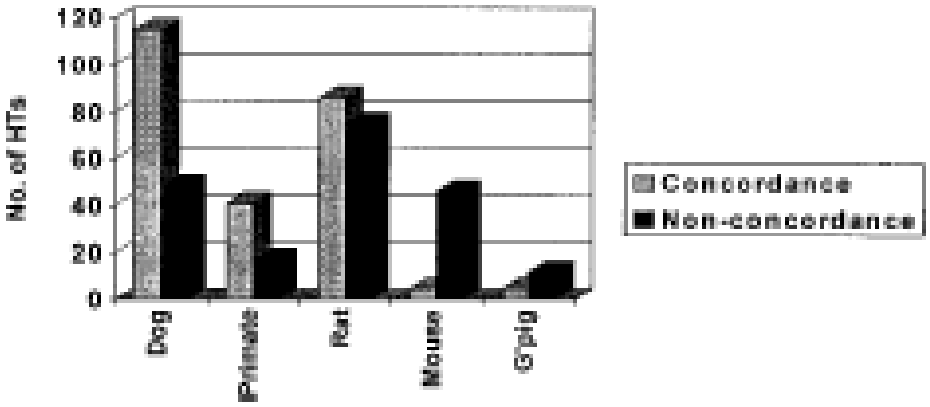


FIG. 4. Concordance rates versus species.

Key factors for good *in vitro* assay

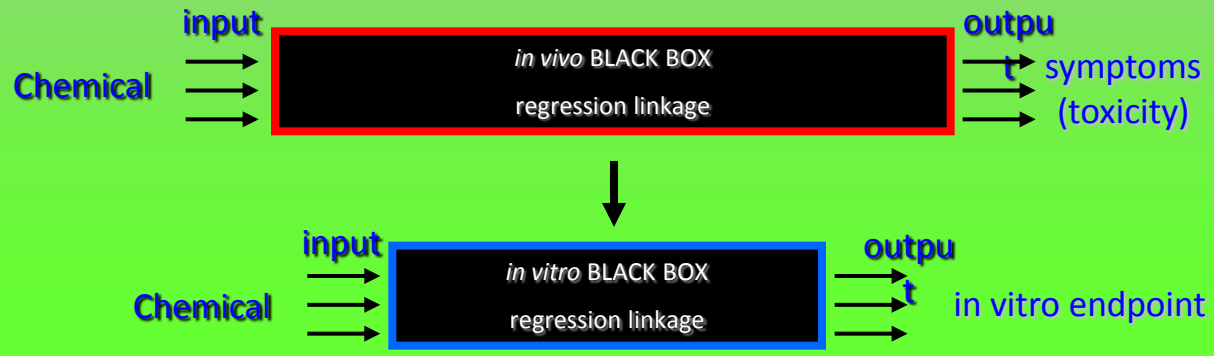
- Test definition based on MoA, WoE
- Valuable Cells
- Optimal biomarker
- High-through put
- Niche (environment) and 3D culture
- Low cost
- 3Rs

Reliability and relevance

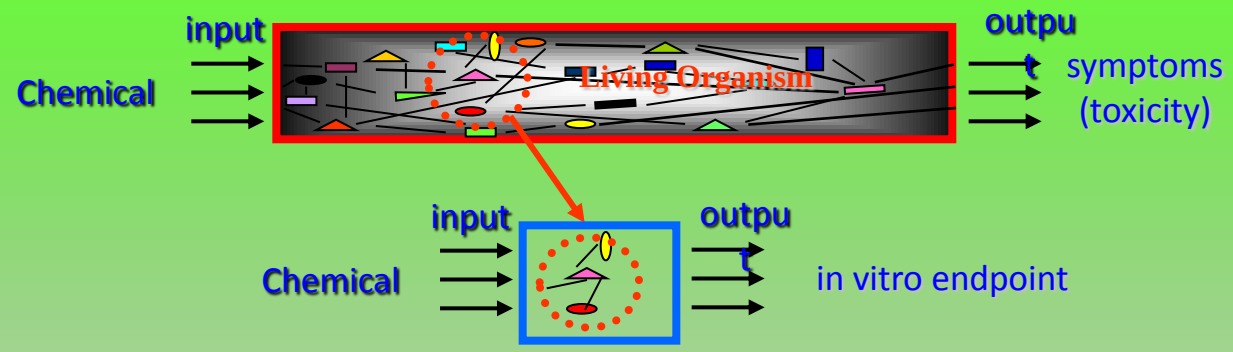
Test definition

Animal model and *in vitro*

(1) Miniature Black Box Approach



(2) *In vivo* mechanism Excision Approach

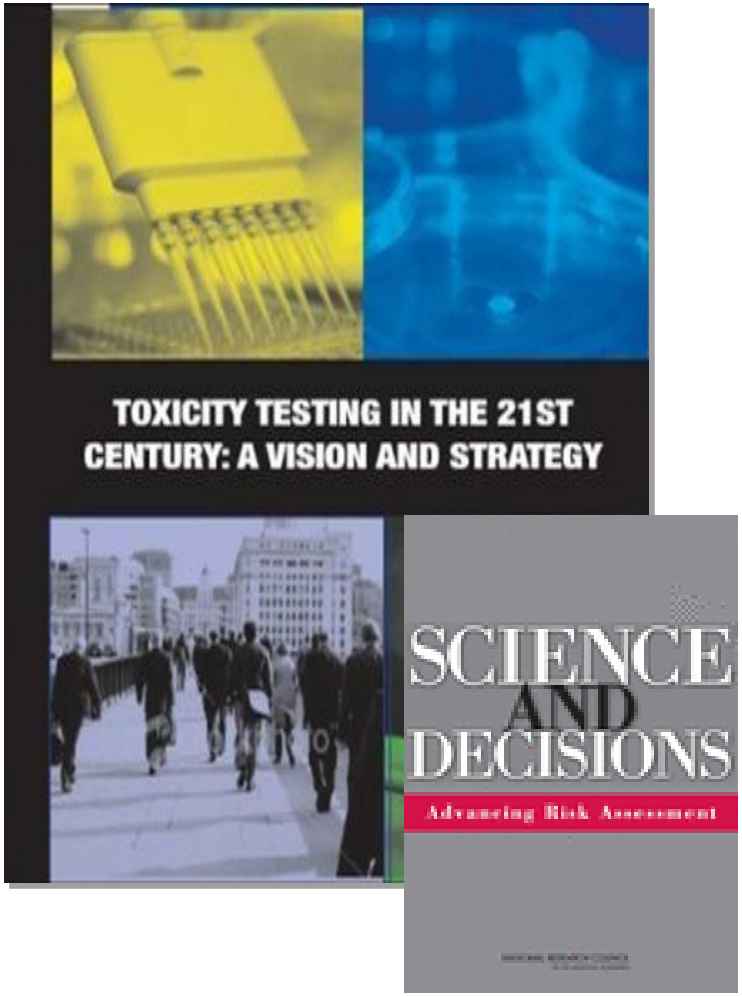


(1) Miniature BlackBox Approach requires "diagnostic" process similar to *in vivo* studies. Until cellular symptoms are well understood to the level of *in vivo* diagnosis, the validation process will be virtually endless.

(2) Mechanism-excision type methods always have Positive controls and Negative controls. With which the validation process is easy and comcise.

By J. Kanno

The NTP Roadmap are consistent with the recent NAS Report



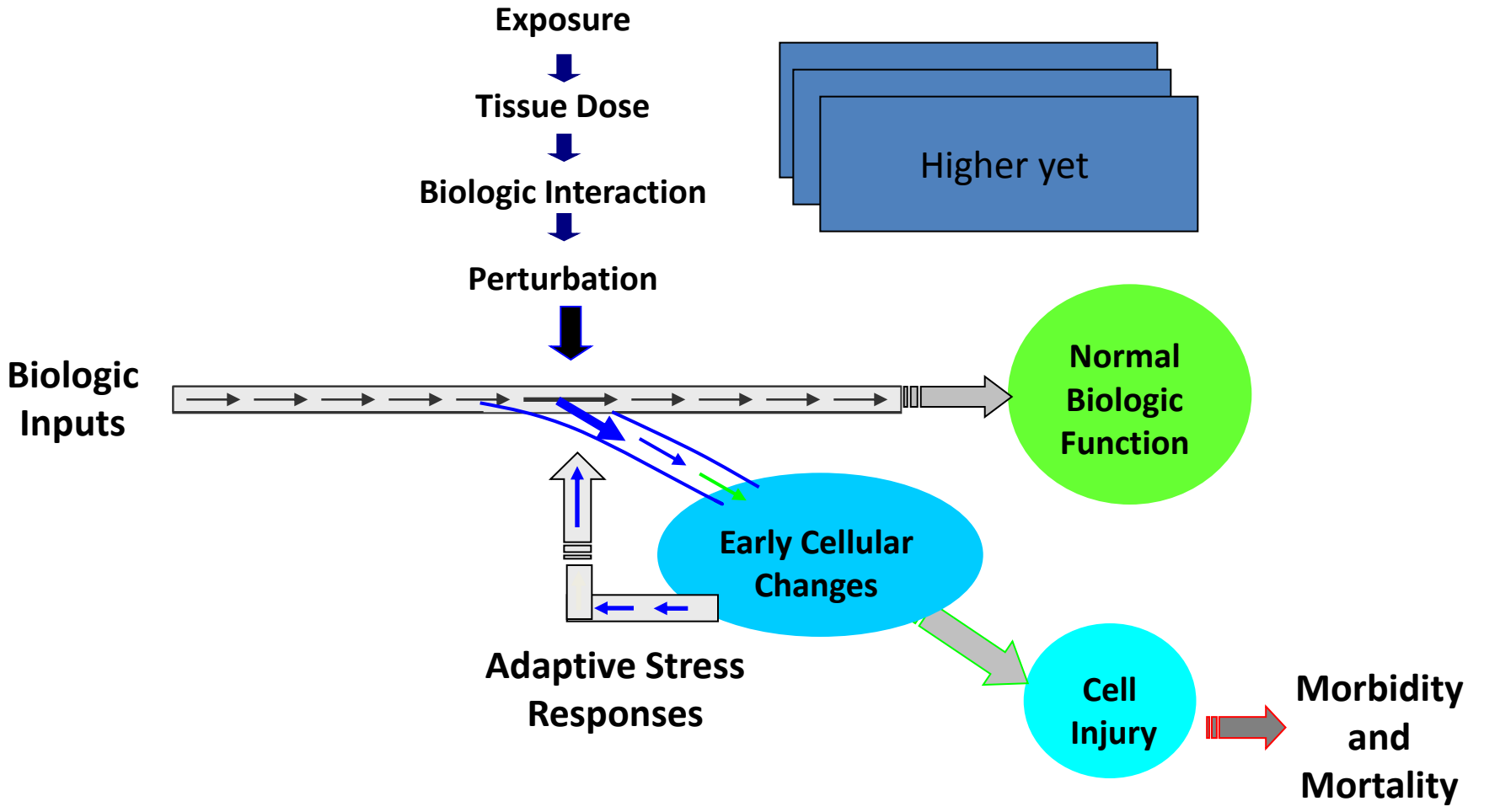
- **2007 NRC Report:**
  - Calls for transforming toxicology: *"from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."*
  - Envisions pathway-based toxicology, where pathway perturbations are used to predict adverse effects
- **2009 NRC report**: *"the realization of the promise [of the 2007 report] is at least a decade away"*

National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Washington, DC: National Academy of Sciences. Available: http://books.nap.edu/catalog.php?record_id=11970

Options for Future Toxicity Testing Strategies

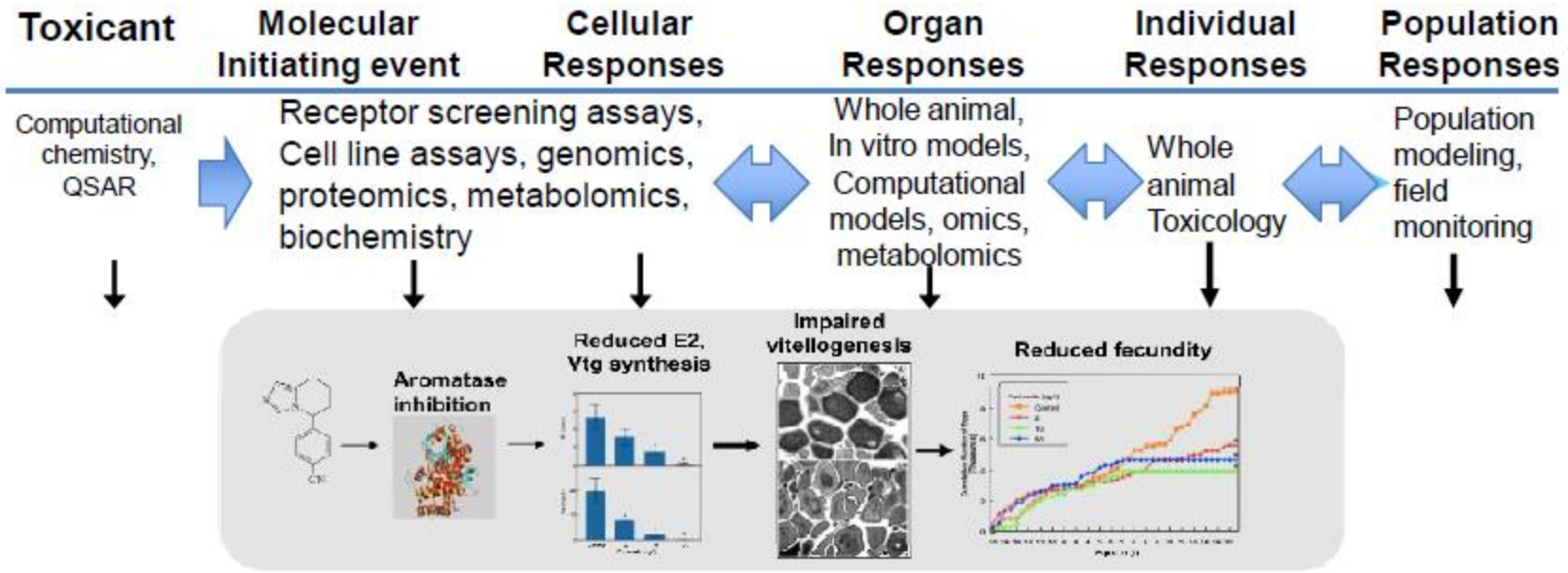
Option I In Vivo	Option II Tiered In Vivo	Option III In Vitro/In Vivo	Option IV In vitro
Animal biology	Animal biology	Primarily human biology	Primarily human biology
High doses	High doses	Broad range of doses	Broad range of doses
Low throughput	Improved throughput	High and medium throughput	High throughput
Expensive	Less expensive	Less expensive	Less expensive
Time consuming	Less time consuming	Less time consuming	Less time consuming
Relative large number of animals	Fewer animals	Substantially fewer animals	Virtually no animals
Apical endpoints	Apical endpoints	Perturbations of toxicity pathways	Perturbations of toxicity pathways
	Some *in silico* and *in vitro* screens	*In silico* screens possible	*In silico* screens

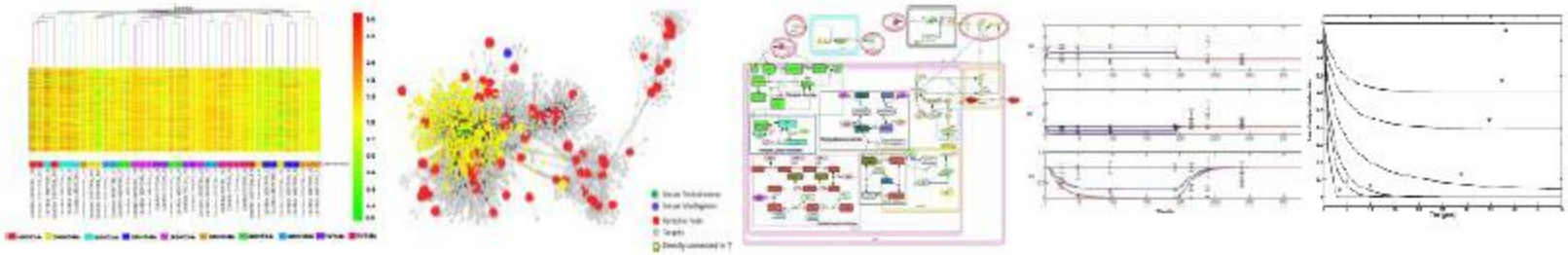
Perturbation of Toxicity Pathways



Adverse Outcome Pathway

AOP and alternative animals in human health assessment





Screening for toxicological effects and chemicals

Pathway and network impacts

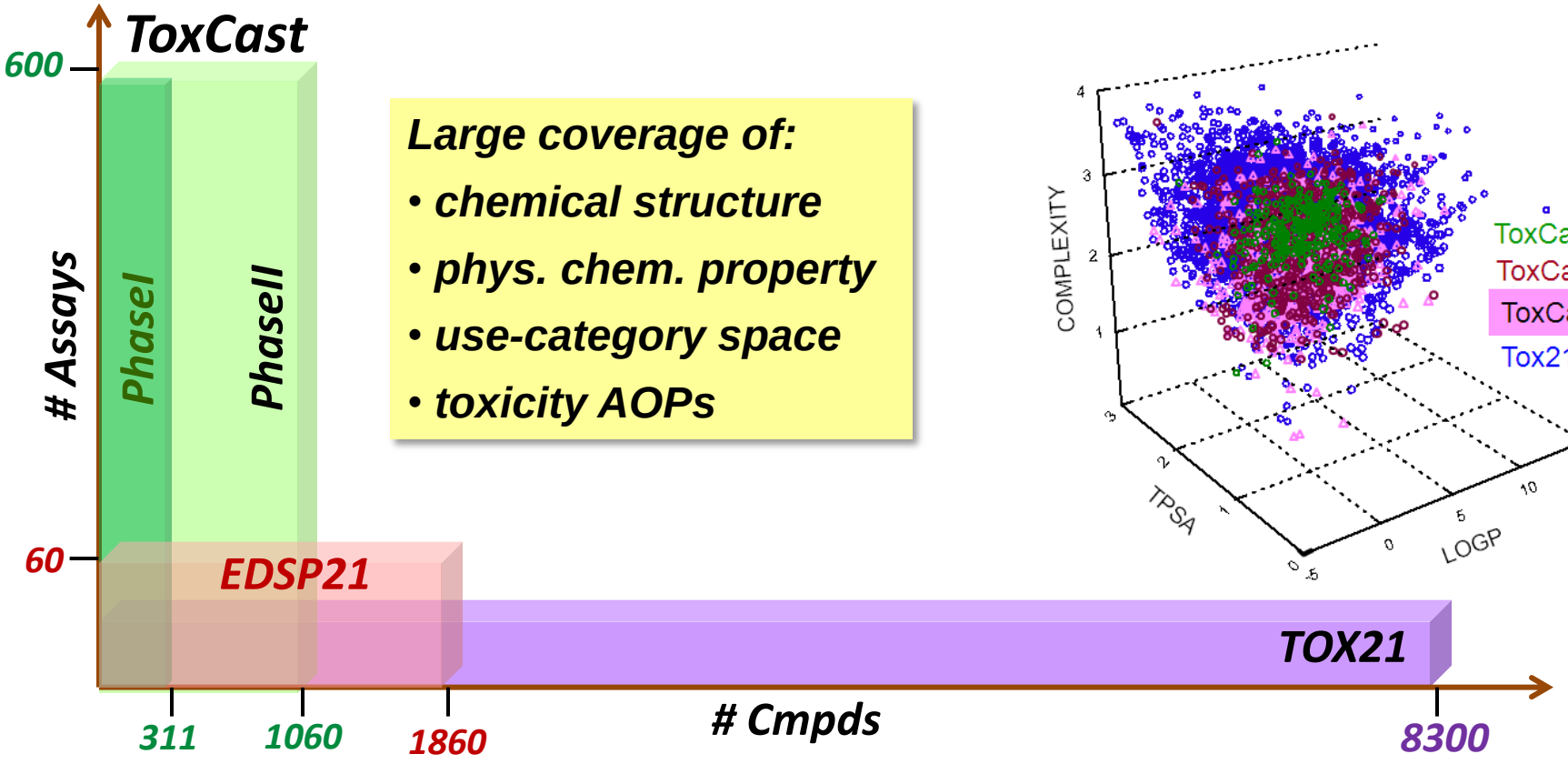
Mechanistic modeling

Predicted effect

Population impact

ToxCast & Tox21 Chemical Inventories

Available for download at: http://www.epa.gov/ncct/dsstox/



Large coverage of:

- ***chemical structure***
- ***phys. chem. property***
- ***use-category space***
- ***toxicity AOPs***

Pesticides, cosmetics and personal care products, fragrances, antimicrobials, food additives, failed drugs, chemicals of concern & green alternatives, industrial HPV & MPV, reference compounds (endocrine, repro/devtox, etc.)

SOURCE: Ann Richard, NCCT

Valuable cells

OECD TG using cell lines (Year 2000 or later)

Method	International acceptance
3T3 NRU Phototoxicity Test	OECD TG 432 (2004)
In vitro Micronucleus assay	OECD TG 487 (2010)
H295R Steroidgenesis assay	OECD TG 456 (2011)
BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists	OECD TG 457 (2012)
Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor AgonistsTest	OECD updated TG 455 (2012)
Fluorescein Leakage (FL) test method	OECD TG 460 (2012)
In vitro skin irritation testing *: reconstructed human epidermis (RHE) test method*	OECD TG updated 439 (2013)
In vitro skin corrosion: reconstructed human epidermis (RHE) test method	OECDTG Updated 431(2013)

Use of human embryonic stem cells for novel toxicity testing approaches

esnats conference together with the EUSAAT congress

European Society for Alternatives to Animal Testing

Afternoon Session			
Chairs	**Time**	**Title**	**Speaker**
antea, IT niversity of Cologne, DE	13:45-14:30	First panel discussion: How can the ESNATS data be used to define mechanisms of action and/or AOPs	Chair: Thomas Hartung, CAAT US-Baltimore - Jan Hengstler, IFADO, DE - Robert Kavlock, EPA, US - Jos KleinJans, University of Maastricht, NL
	14:30-15:00	Enhancing the readout of the embryonic stem cell test with molecular approaches	Aldert Piersma, RIVM, NL-Bilthoven
	15:00-15:30	Interspecies comparison of pathways contributing to neurodevelopmental toxicity: Neurospheres as test systems which model processes involved in brain development	Ellen Fritsche, Leibniz Research Institute for Environmental Medicine, DE-Düsseldorf
	15:30-16:00	Transforming the Conduct of Toxicology in the US : the Tox21 Program	Robert Kavlock, US Environmental Protection Agency, US-Washington
	16:00-16:30	*Coffee break and posters*	
	16:30-17:00	Predictive models and computational embryology	Thomas Knudsen, US Environmental Protection Agency, US-Research Triangle Park
	17:00-17:30	EPAA calls for a "Stem Cells in Safety Testing" forum to keep fluent communication	Beatriz Silva-Lima, EPAA stem cell group, BE-Brussels
	17:30-18:00	Data infrastructure for chemical safety	Jos Kleinjans, University of Maastricht,

Assays for Hand1-luc EST

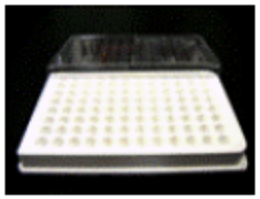
Hand1-ES (KOB1) cells

Test compounds

Day 0

Day 6

Differentiation assay

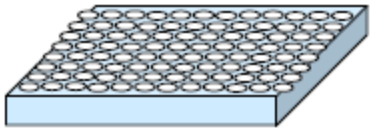


white U96 well plate

Luc-activity (luminescence)

ES-ID50 (Differentiation toxicity)

Cytotoxicity assay



96well plate

Cell viability assay (luminescence)

ES-IC50 (ES/cytotoxicity)

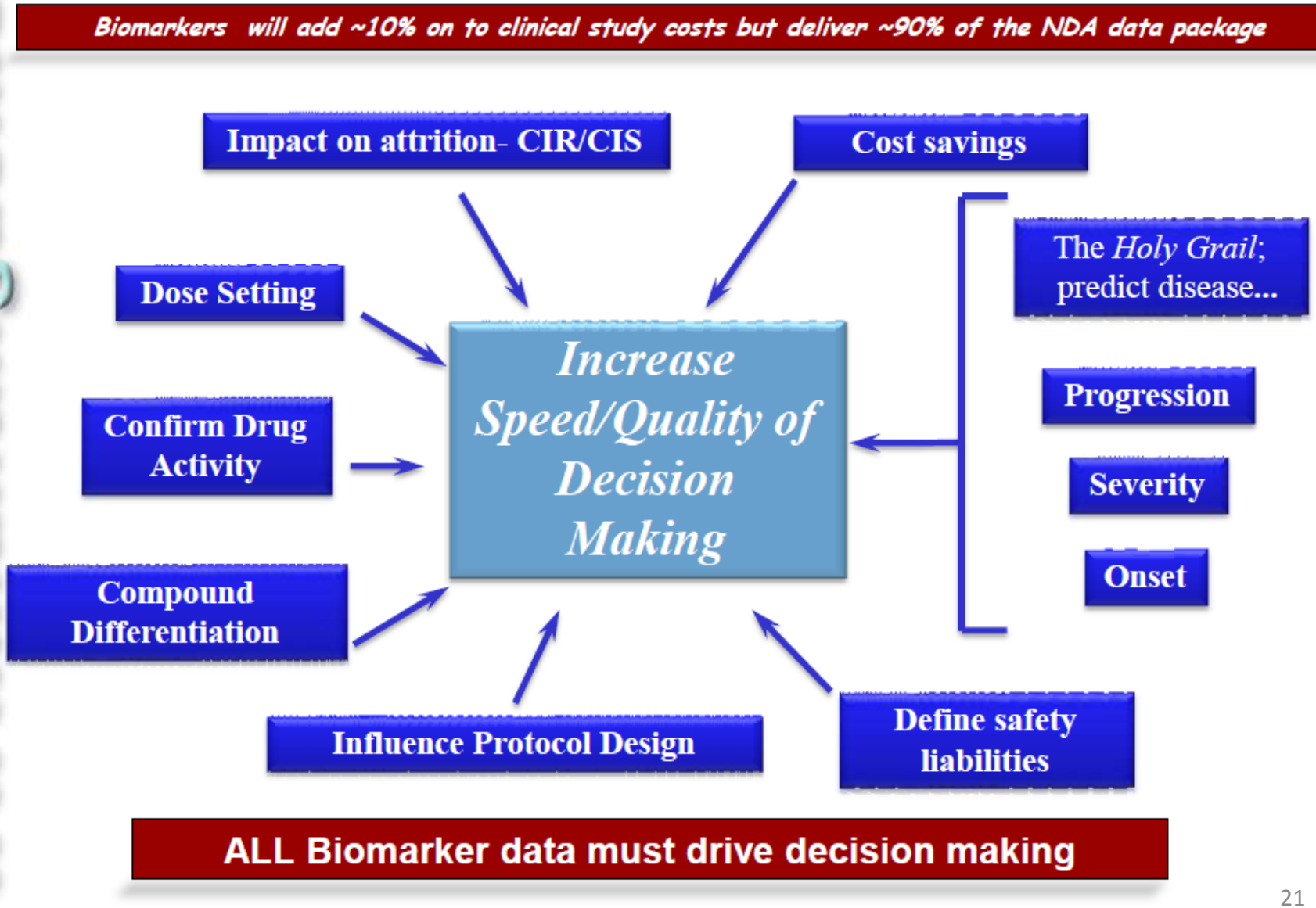
Optimal biomarker

E15 Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories

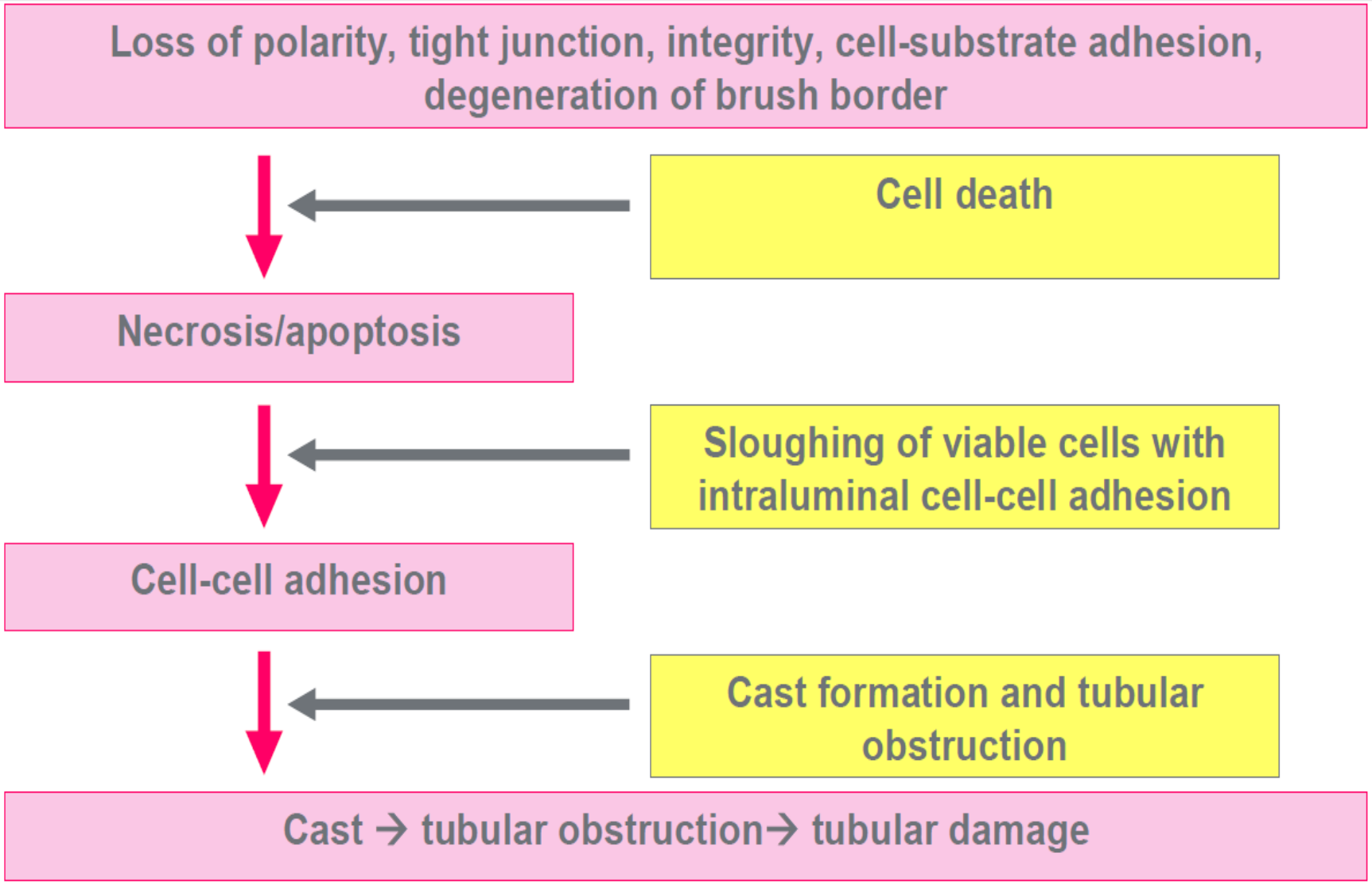
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

April 2008
ICH

Biomarkers and their Value Proposition



Progression of Tubular Damage



シンポジウム（Symposium）3

9：30～12：00

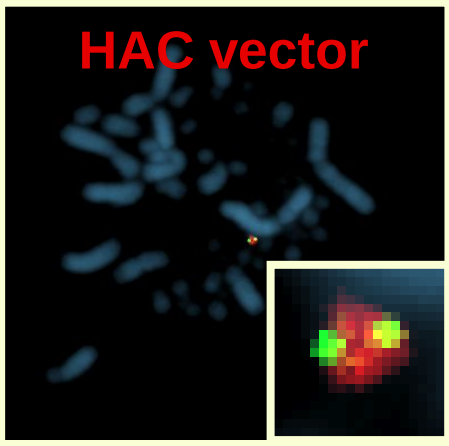
薬物動態・毒性研究におけるバイオマーカーの選択と応用
Toxicology DIS Part 1, Biomaker Discovery and Evaluation in DMPK and Toxicology Studies

Organizer・Chairs:	横井　毅	名古屋大学大学院医学系研究科　統合医薬学領域トキシコゲノミクス
	Tsuyoshi Yokoi	Department of Drug Safety Sciences, Nagoya University Graduate School of Medicine
Chairs:	堀井　郁夫	ファイザー株式会社
	Ikuo Horii	Pfizer Japan Inc.

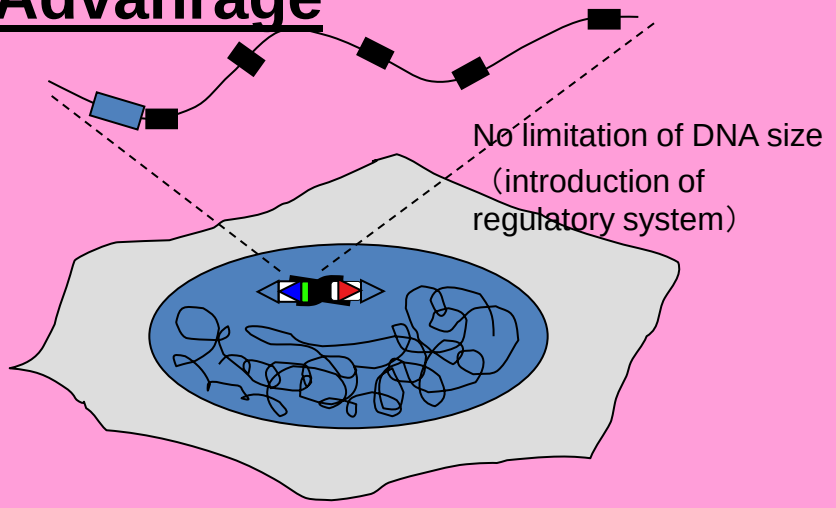
High-through put

Human Artificial Chromosome vector

Novel technology for gene transfer into cells



Advanrage



- No integration in genomic DNA
- One copy and stable
- Physiological regulation
- No over-expression/no silencing

(1) High quality

→Validation, development of guidelines

Expression of transgenes in a consistent manner

Easy to insert genes (reduction in time)

(2) Efficient analysis

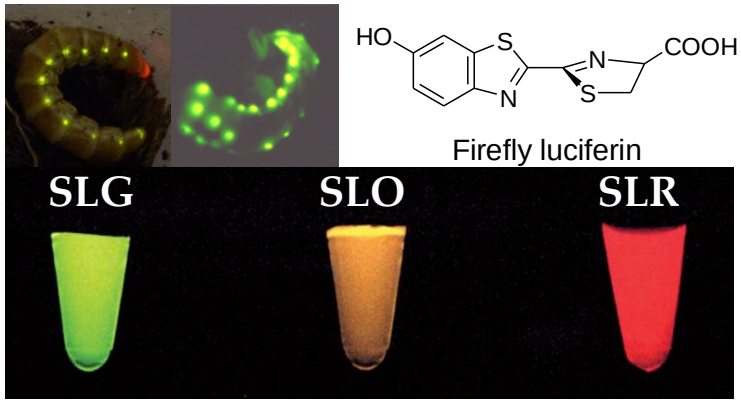
→ Transfer of multiple genes

Multi-color bio-imaging

Multicolor and secretion luciferase assay system

An effective screening system

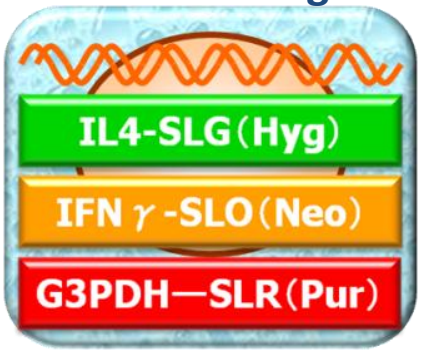
Simultaneous monitoring of 3 gene expressions

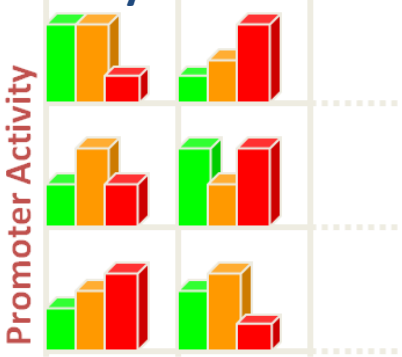


Firefly luciferin

・Multiple gene transcription activity assay system.
JP4385135, US7572629, CN1784496, EP1784496

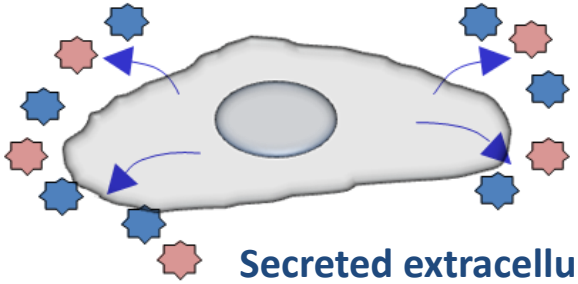
The system can measure promoter activities of two targets genes and one internal control gene in HTP system.





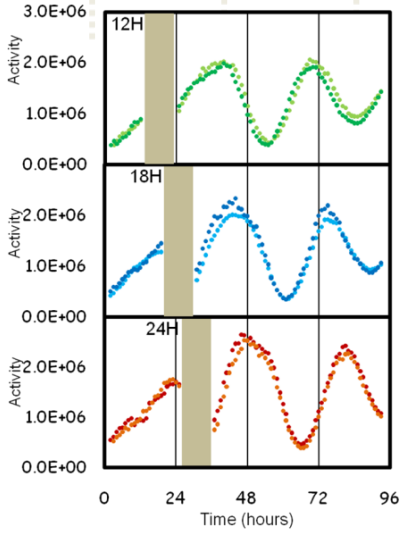
Monitoring of 2 gene expressions in secreta

Secretion luciferases GLuc & CLuc



Secreted extracellularly

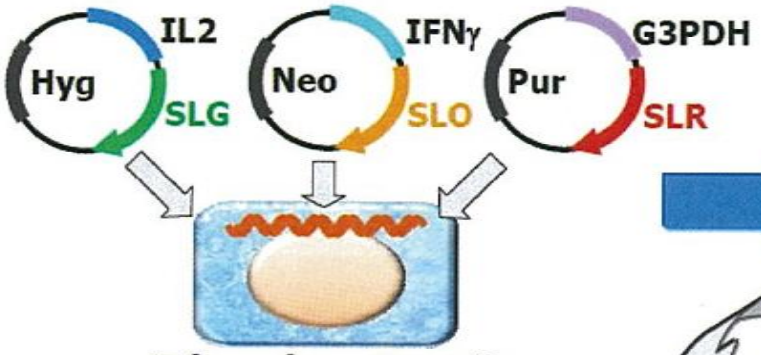
It can measure luciferase activities in culture medium (in vitro), blood or urine (in vivo).

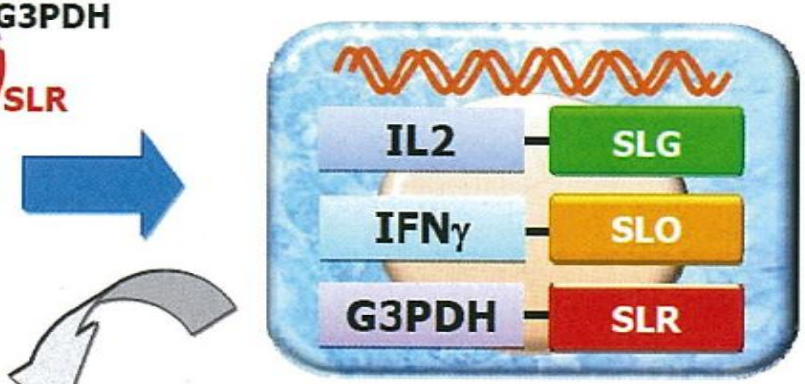


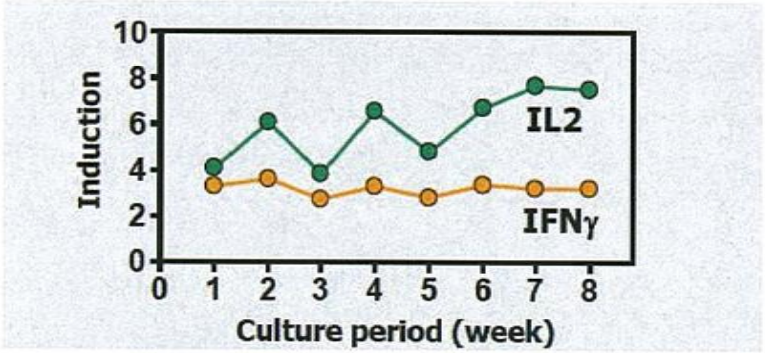
・Luciferin luminescent substrate of marine ostracod crustacean and method for production thereof.
JP4915955, US7989621, US8343729

Example of toxicity test for immunology using a multireporter assay

Generation of T cells stably express SLG, SLO and SLR enzymes under two marker gene promoters and internal control gene promoter.



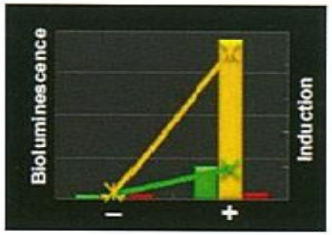




Cells display stable luciferase expressions and respond to chemicals during prolonged culture.

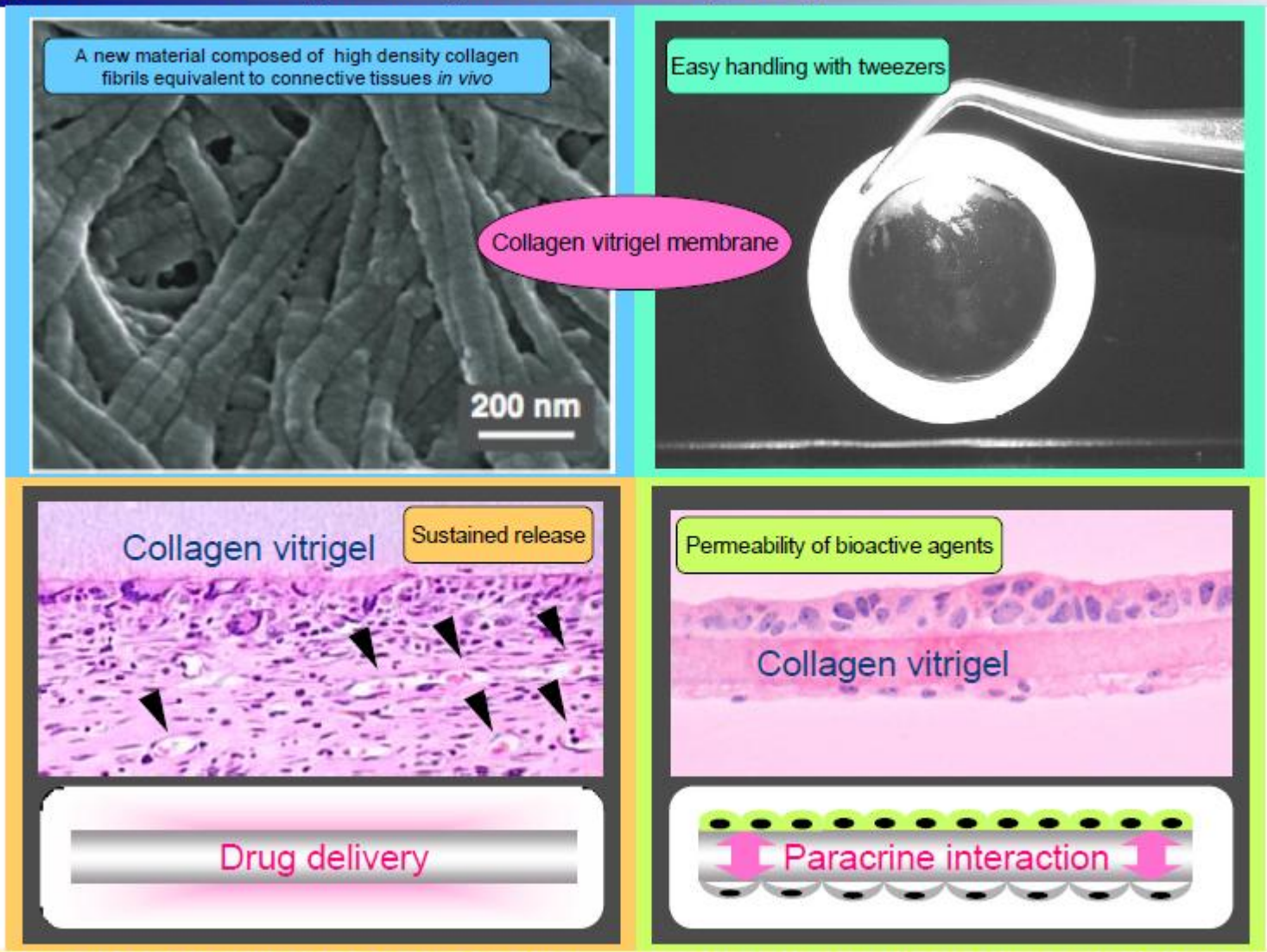
Chemical risk analysis in a 96well plate format HTP assay

Reaction with Tripluc® assay Reagents



Niche (environment) and 3D culture

Background– 1 : Collagen vitrigel membrane (CVM)



Takezawa T, *et al.*, Cell Transplantation, 13: 463–473, 2004
Takezawa T, *et al.*, Tissue Engineering, 13: 1357–1366, 2007

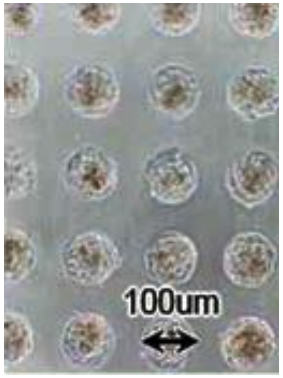
Takezawa T, *et al.*, Cell Tissues Organs, 185: 237–4241, 2007
Takezawa T, *et al.*, Yakugaku Zasshi, 130: 565–574, 2010

Cell Culture Technique

(three dimensional culture / stem cell differentiation)

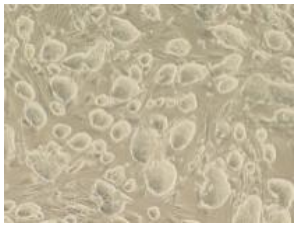
Liver

Liver Spheroids

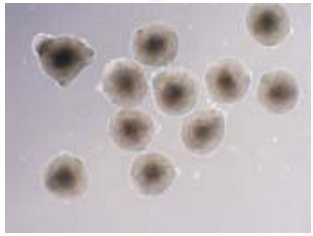


Three dimensional culture method (Cell able, transparent Inc.)

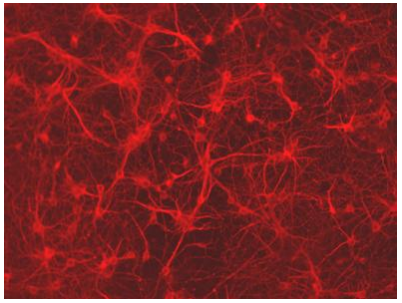
ES cell (mouse)



differentiation

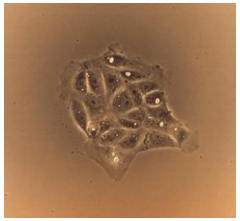


Nerve



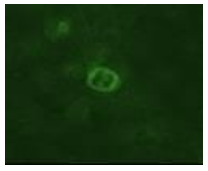
Kidney

rat adult kidney stem / progenitor like cells (rKS cells)

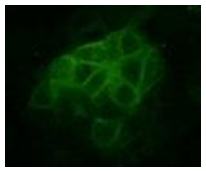


Aquaporin-1

Undifferentiated



Differentiated



(Kitamura S et al. FASEB J, 2005)

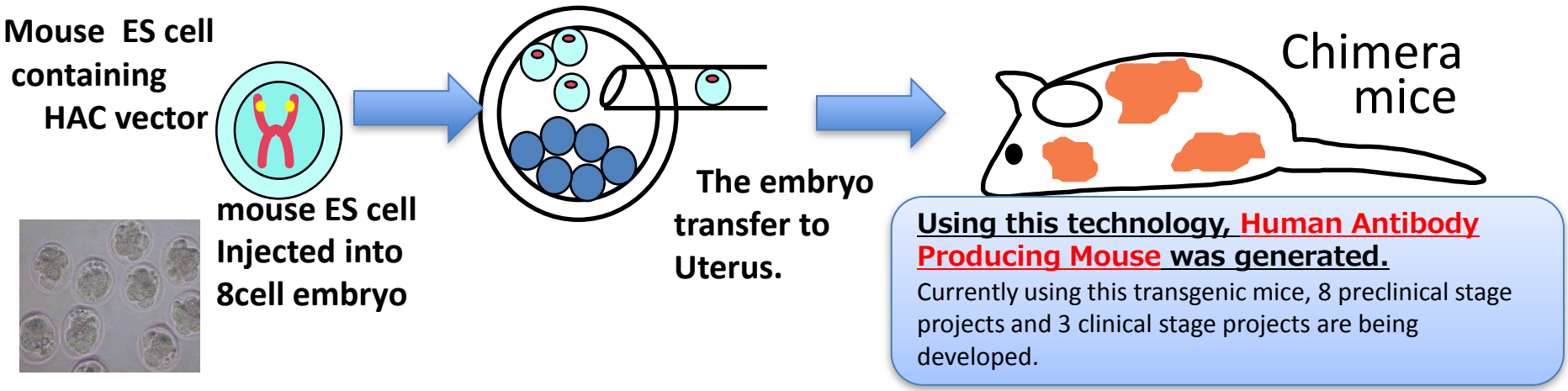
Kidney-like structure (*In Vitro*)

Transgenic mice

Relationships between *in vitro* and *in vivo* response

→**Trans-chromosomic mice**: Novel Transgenic mice using

「Chromosome engineering」& 「Developmental engineering」



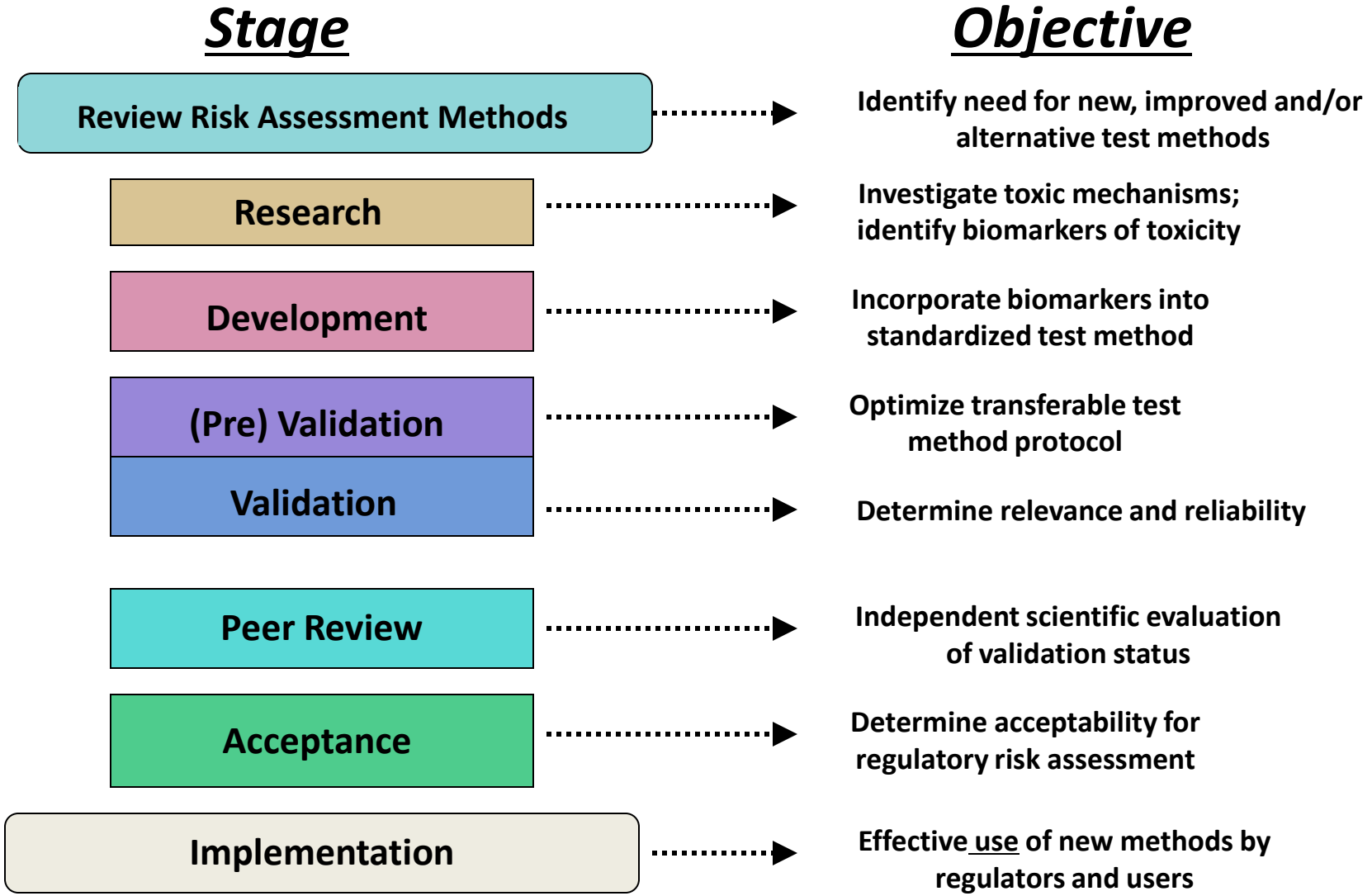
Creation of trans-chromosomic Chimera mice with the HAC vector

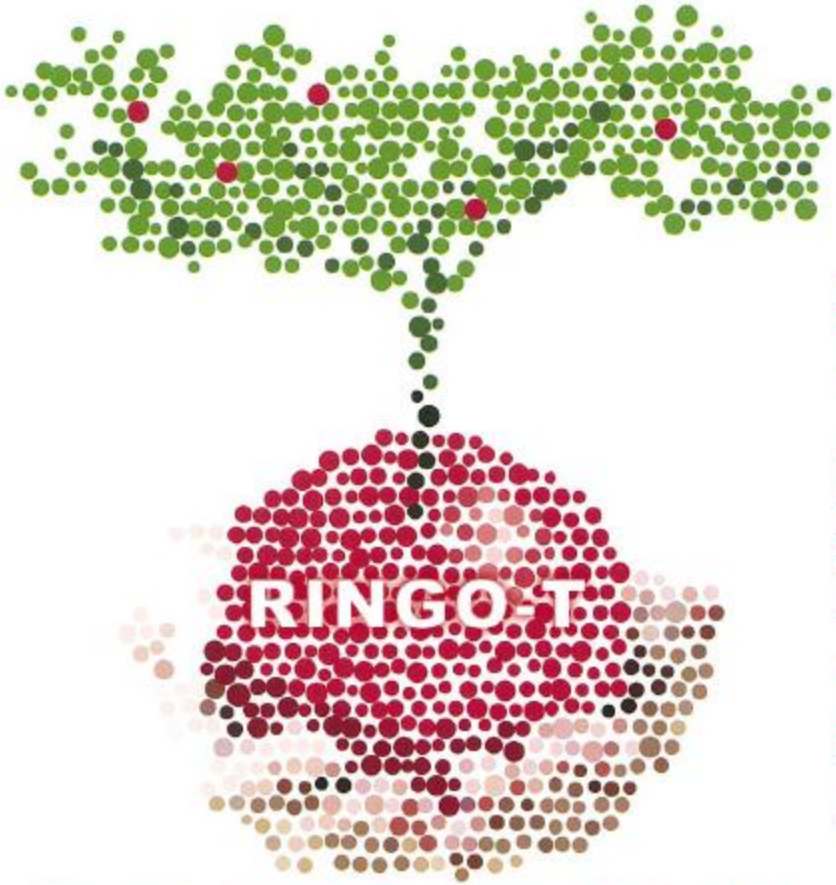
In vitro generation of organs derived from pluripotent stem cells is very difficult since it requires reproducing various types of different component cells and three-dimensional structures

→Target tissues (or cells) can be created using embryonic development within individual body.

Reliability and relevance

Test Method Evolution and Translation Process: Concept to Implementation





求ム、革新への挑戦。

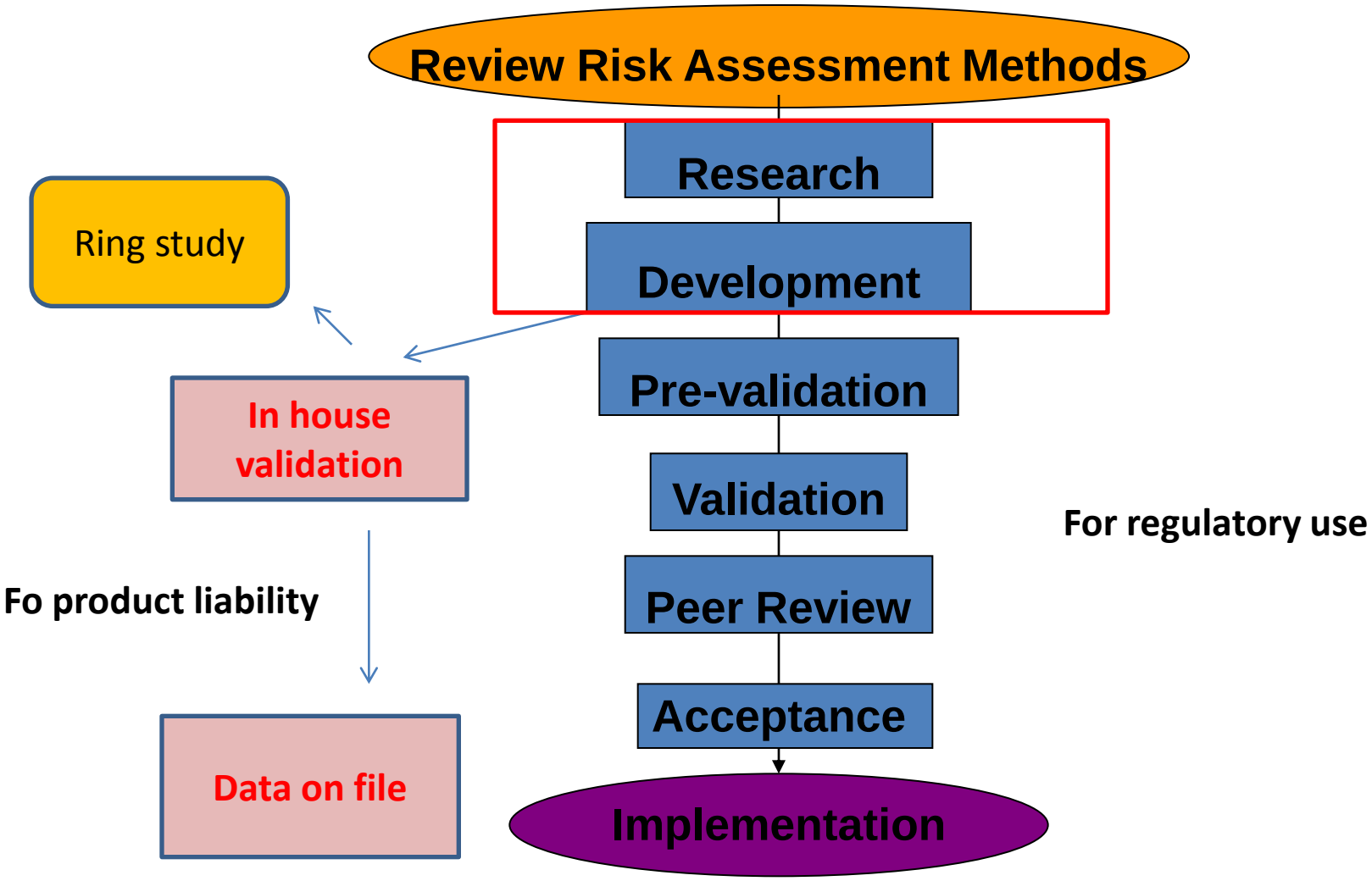
RINGO-T 2013

Recruit Innovative Ideas to Generate Original Targets Takeda

タケダ オープンイノベーション 2013

武田薬品は、「優れた医薬品の創出を通じて人々の健康と医療の未来に貢献する」というミッションのもと
優れた新薬を創出するため「革新への挑戦」を追求しています。
この目的を達成するためアカデミアからユニークな細胞アッセイを募集致します。

Test method validation process



Summary

In vitro test methods for screening safety and efficacy of new drugs contribute to lower development costs, more accurate predictions of side effects, and increased animal welfare. There are several newly developed assays based on mechanisms of action (MoA) and preclinical biomarkers that meet the need for simple and easy-to-use test methods capable of high throughput and offering good intra-laboratory repeatability.

Japanese Center for the Validation of Alternative Methods

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

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About JaCVAM


Update on JaCVAM


Academic activities


Submission of Alternative Methods to JaCVAM


International Cooperation

Thank you for your 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）

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