January 20 (US)/21(Asia), 2022
Virtual Training Workshop

USP-APEC Center of Excellence for Advanced Therapies
“Development and Validation of Bioassays for Advanced Therapies”

Regulatory Perspectives on Good Practices and Guidances that Support Bioassays for Characterization of Cell Therapy Products

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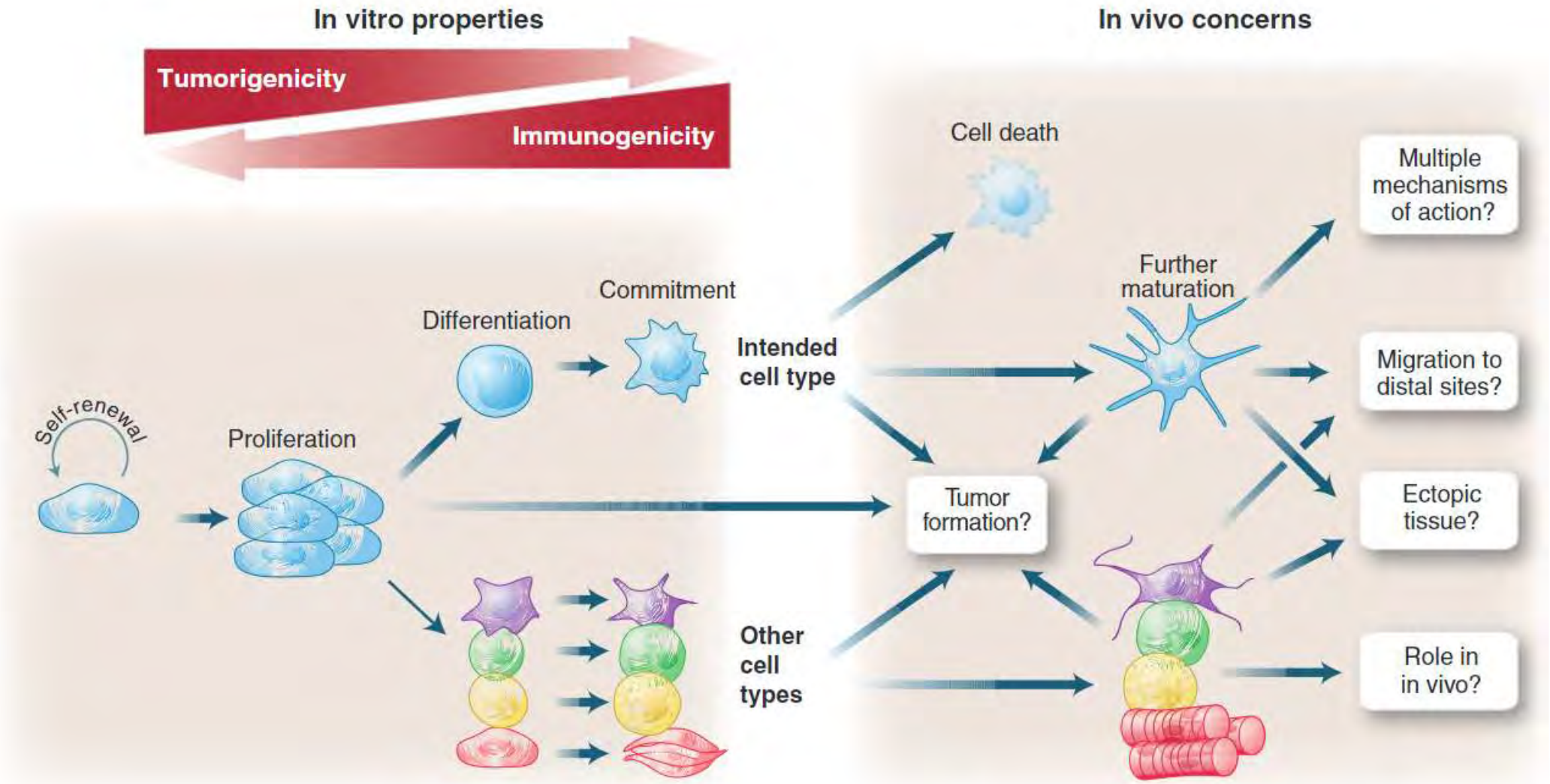
DISCLAIMER

The views and opinions expressed in this presentation are those of the presenter and do not necessarily represent official policy or position of the Japan National Institute of Health Sciences or the Japan Ministry of Health, Labour and Welfare. Also, the presenter has no COI to disclose in connection with this presentation.

Challenges Specific for Cell Therapy Products (CTPs)



Fink DW Jr. Science. 2009;324:1662-3.

CTPs are more complex than conventional biologics

Cells are complex = dynamic "living" systems

- Cell traits depend on the (micro)environment in which they are placed.
  - Species specific (i.e., difficult to assess safety of human cells in xenogenetic environment (non-clinical studies))
  - Pathology specific (e.g., normal vs. ischemic/inflammatory environment)
- Cells also act on their surrounding environment pharmacologically, immunologically, physically, etc.
- Cells may de-differentiate or their traits may be changed during long-term culture
- Cells may migrate, posing challenges in pharmacokinetics and biodistribution studies
- Cells are fragile and have a finite lifespan, which poses issues about transport and shelf-life
- Difficult to highly purify active cellular ingredients and to inactivate/remove virus in the products

Cell characterization is critical.

Traditional quality control, non-clinical and clinical trial methods may not always be applicable.

High diversity among products

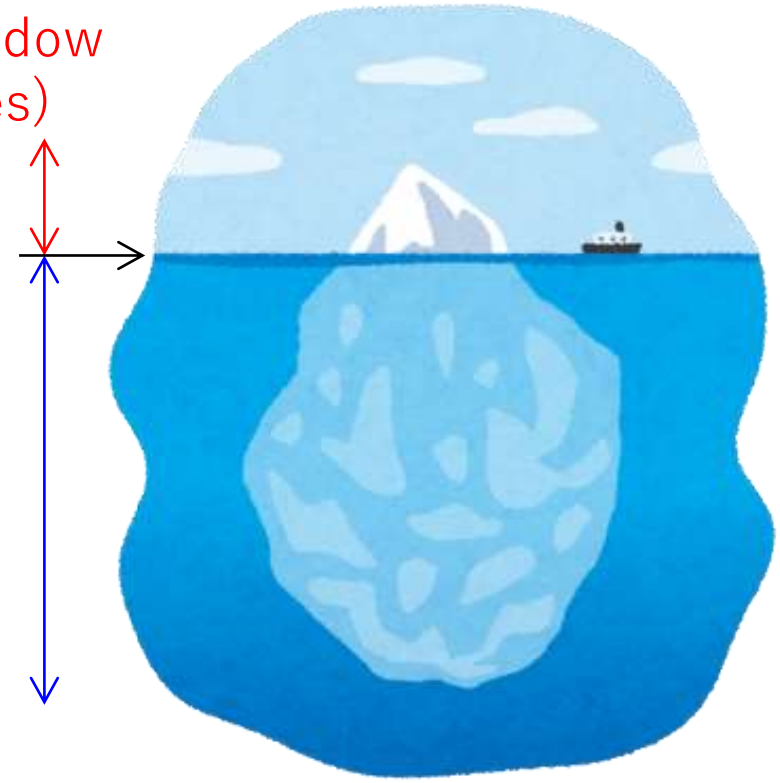
- Various risk sources and impacts make it important to ensure effectiveness, safety, and quality through a case-by-case and risk-based approach.

CTPs are more complex than conventional biologics

Limited Characterization Window
(Observable Quality Attributes)

Limit of Knowledge

Unobservable
(but Potentially Critical)
Quality Attributes



Efficacy

The mode of action (MOA) is unclear in many cases.

Technical Issues on Bioassays (Potency Assays) for CTPs

- Selection of bioassays (potency assays) in cases where the mechanism of action is unclear
- Validity and reliability of analytical methods, including bioassays (potency assays), in cases where their characteristics are not well validated
- Lack of reference standards

Technical Issues on Bioassays (Potency Assays) for CTPs

- Selection of bioassays (potency assays) in cases where the mechanism of action is unclear

"Specifications will differ among final products, depending on the type and properties of the desired cells and tissues, manufacturing methods, intended clinical use, method of clinical application, stability, and test methods.

…(omission)… The purpose of the assessment at the initiation of clinical trials is to confirm that the product in question is unlikely to pose significant quality/safety problems for use in investigational clinical trials.

Therefore, **it is possible to set provisional specifications with allowance for some variation on the basis of the measured values obtained for a few test specimens, as long as one can explain about the relationship between the results of clinical tests and the quality attributes after the clinical trial.**

…(omission)… **It should be noted that the quality control strategy, including specifications, should be enriched and developed as the clinical trial progresses.**"

Five guideline documents on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of human stem cells (Notifications No. 0907-2 to 0907-6, issued by Pharmaceuticals and Food Safety Bureau, Japan Ministry of Health, Labour and Welfare on September 7, 2012.) https://www.sciencedirect.com/science/article/pii/S2352320415000176 https://www.sciencedirect.com/science/article/pii/S2352320415000164 https://www.sciencedirect.com/science/article/pii/S2352320415000218 https://www.sciencedirect.com/science/article/pii/S2352320415000231 https://www.sciencedirect.com/science/article/pii/S2352320415000206

Technical Issues on Bioassays (Potency Assays) for CTPs

- Selection of bioassays (potency assays) in cases where the mechanism of action is unclear

In many cases, it is difficult to clearly identify critical quality attributes that are involved in the efficacy or safety of a cell therapy product.

Therefore, **it is important that specifications (test procedures and acceptance criteria)**, as well as process parameters and in-process control tests, **include items for controlling potential quality attributes that are assumed to have a significant impact on the efficacy or safety of the product** through quality risk assessment based on an understanding of the manufacturing process **and are very likely to be real critical quality attributes.**

A quality control strategy for a cell therapy product may be acceptable, if the verification plan proposed by the sponsor is considered to produce a product with consistent quality at each manufacture as at the time of manufacture of the investigational product.

Review Report of HeartSheet (https://www.pmda.go.jp/regenerative_medicines/2015/R20151008001/470034000_22700FZX00002_A100_2.pdf [in Japanese], Office of the Counselor for Medical Devices and Regenerative Medical Products, Pharmaceuticals and Food Safety Bureau, Japan Ministry of Health, Labour and Welfare on September 2, 2015.) translated by Yoji Sato with slight modifications

Technical Issues on Bioassays (Potency Assays) for CTPs

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"Specification"

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]

- **"Specification"** is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described.

- **Specifications** are one part of a total control strategy designed to ensure product,

- … and chosen to confirm the quality of the drug substance and drug product rather than to establish full characterization.

Characterization of a biotechnological or biological product (which includes the determination of physicochemical properties, **biological activity**, immunochemical properties, purity and impurities) **by appropriate techniques is necessary to allow relevant specifications to be established**.

Analytical Techniques → Product Characterization → Specifications

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]

Analytical Techniques for determination of

Physicochemical Properties, **Biological Activity**, Immunochemical Properties, Purity & Impurities

"Bioassays (Potency Assays)"
… **need to be validated** , for which an appropriately characterized (in-house) **reference standard** should be established.

Product Characterization → **Extensive characterization needs to be performed**

1) in the development phase and,

2) where necessary, following significant process changes.

Product Specifications

Analytical Techniques → Product Characterization → Specifications

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]

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↓

Product Specifications

"Potency"

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]

- Potency (expressed in units) is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties.
- Mimicking the biological activity in the clinical situation is not always necessary. A correlation between the expected clinical response and the activity in the biological assay should be established in pharmacodynamic or clinical studies.

This is difficult for "advanced" therapies, of which no one has clinical experience.

Variety of CTPs means that potency assays (bioassays) for them are product-specific or class-specific, not "one-size-fits-all".

Analytical Techniques → Product Characterization → Specifications

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Product Specifications

"Reference Standard"

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]

- **The results of biological assays should be expressed in units of activity calibrated against an international or national reference standard, when available and appropriate for the assay utilized.**

- **Where no such reference standard exists, a characterized in-house reference material should be established and assay results of production lots reported as in-house units.**

In potency assays (bioassays) for CTPs, it is difficult to obtain a well-established reference standard.

Analytical Techniques → Product Characterization → Specifications

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Product Specifications

"Bioassay (Potency Assay)"

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]

- *"Biological activity"* describes the specific ability or capacity of a product to achieve a defined biological effect.

A matrix of bioassays may be required because a single assay may not provide sufficient information to predict the efficacy of a CTP.

- Valid biological assays (also called potency assays or bioassays) to measure the biological activity should be provided by the manufacturer, e.g.,

a. Animal-based biological assays, which measure an organism's biological response to the product
   (animal death, survival, tumor shrinkage etc.)
b. Cell culture-based biological assays, which measure biochemical or physiological response at the cellular level
   (cell growth, cell death, motility, differentiation, infectivity, transgene product, etc.)
c. Biochemical assays, which measure biological activities such as enzymatic reaction rates or biological responses induced by immunological interactions.
   (release of cytokine/growth factor/exosomes from the product, etc.)

Analytical Techniques → Product Characterization → Specifications

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Product Specifications

"Validation of Analytical Procedures"

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- At the time the application is submitted to the regulatory authorities, applicants should have validated the analytical procedures used in the specifications in accordance with the ICH Harmonised Tripartite Guidelines (ICH Q2(R1)):

"Text on Validation of Analytical Procedures: Definitions and Terminology"

and

"Validation of Analytical Procedures: Methodology"

ICH Q2(R1) : VALIDATION OF ANALYTICAL PROCEDURES

Validation characteristics regarded as the most important for the validation of different types of analytical procedures

Type of analytical procedure / characteristics	IDENTIFICATION	TESTING FOR IMPURITIES		ASSAY - dissolution (measurement only)	ASSAY - content/potency
		quantitat.	limit		
Accuracy	-	+	-		+
Precision					
Repeatability	-	+	-		+
Interm.Precision	-	+ (1)	-		+ (1)
Specificity (2)	+	+	+		+
Detection Limit	-	- (3)	+		-
Quantitation Limit	-	+	-		-
Linearity	-	+	-		+
Range	-	+	-		+

- signifies that this characteristic is not normally evaluated

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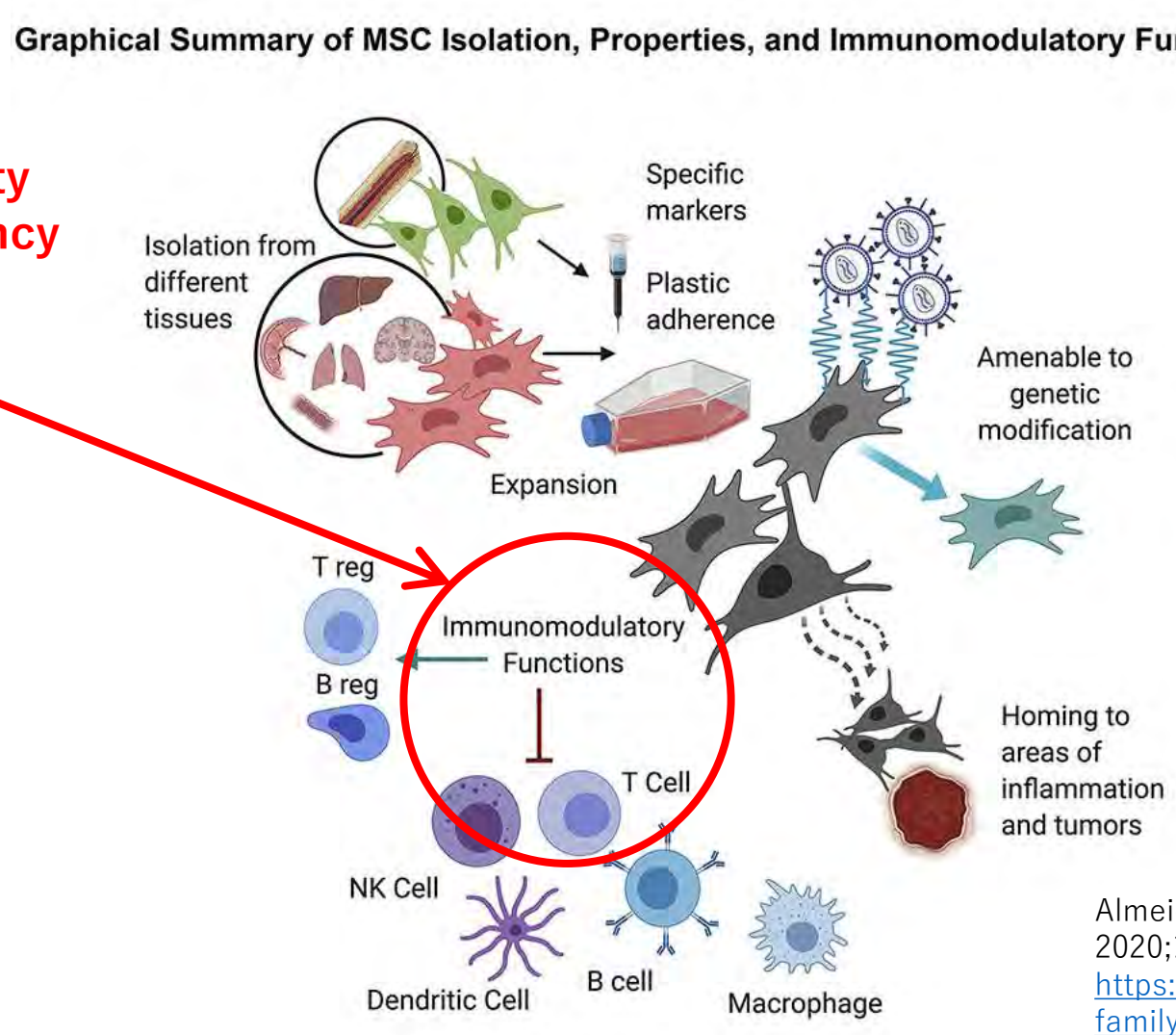
(3) may be needed in some cases

— Case Study on Validation of a Potency Assay —

Mixed Lymphocyte Reaction Assay for Mesenchymal Stem/Stromal Cells (MSCs)

EXAMPLE:
The biological activity relevant to the potency assay results



Almeida-Porada G, et al., Mol Ther Methods Clin Dev. 2020;16:204-224.
https://www.cell.com/molecular-therapy-family/methods/fulltext/S2329-0501(20)30016-4

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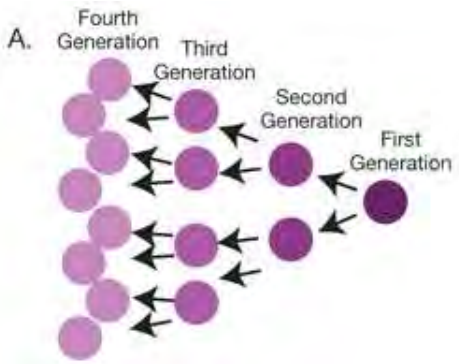
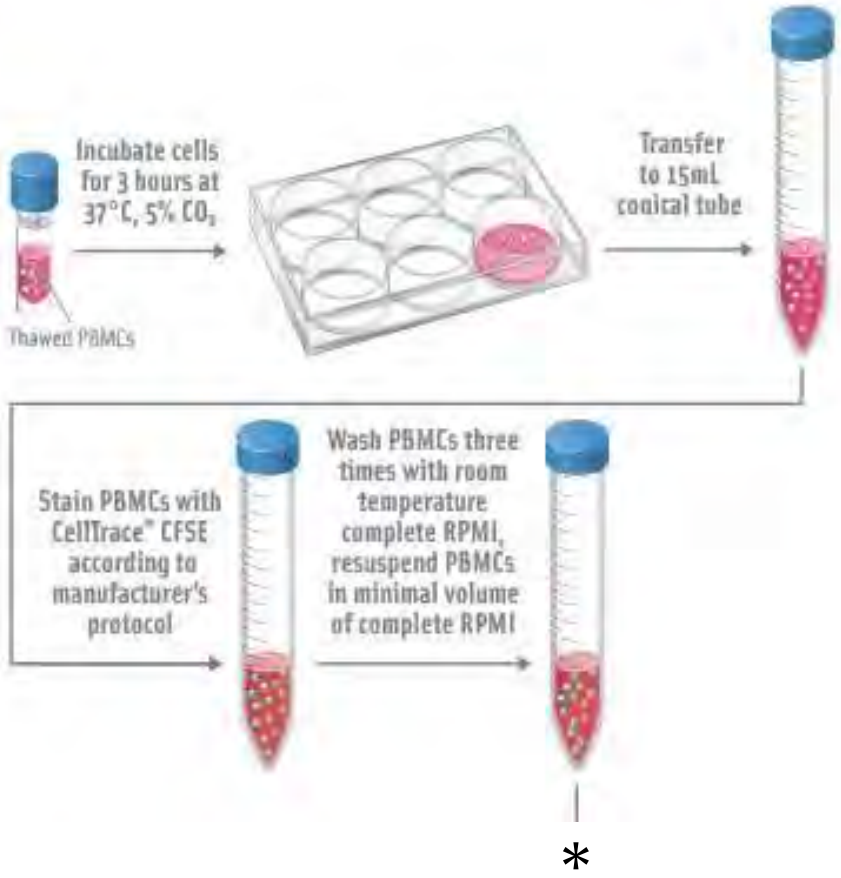
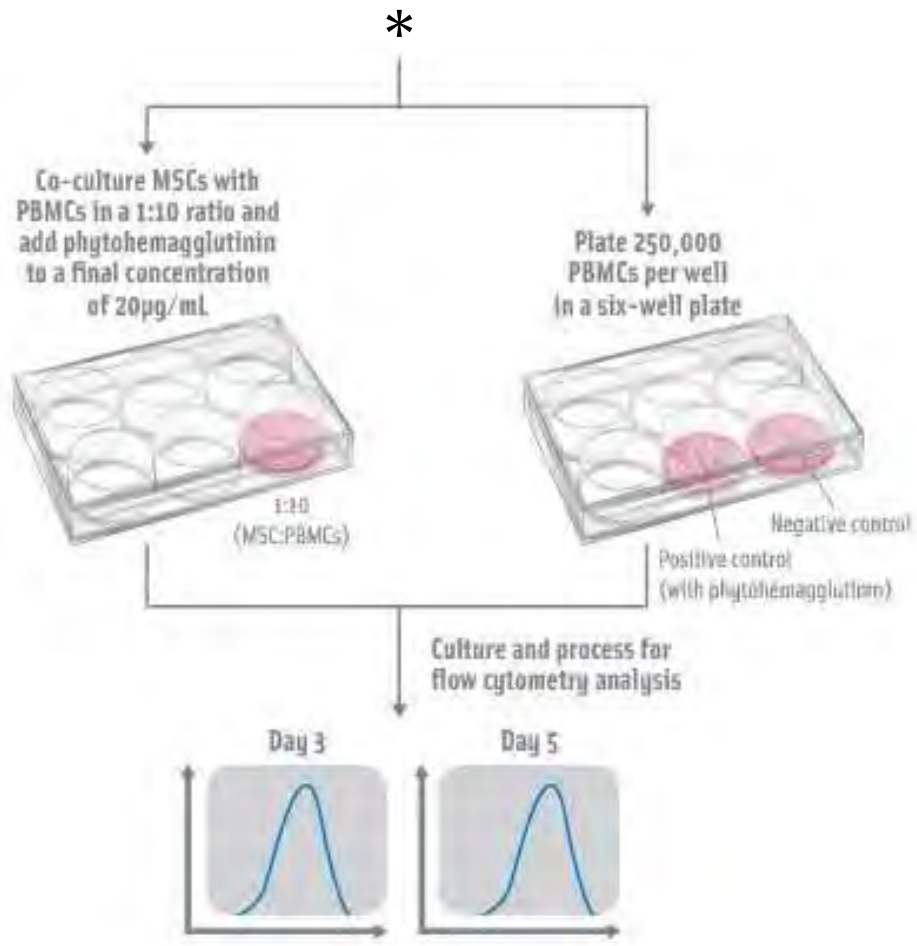
Mixed Lymphocyte Reaction Assay for Mesenchymal Stem/Stromal Cells (MSCs)

CellTrace™







PBMCs: Peripheral blood mononuclear cells

https://www.thermofisher.com/jp/ja/home/life-science/cell-analysis/flow-cytometry/flow-cytometry-assays-reagents/cell-proliferation-flow-cytometry/improved-cfse-alternatives-cell-proliferation.html

https://www.irvinesci.com/protocol-for-mesenchymal-stem-cell-immune-modulation

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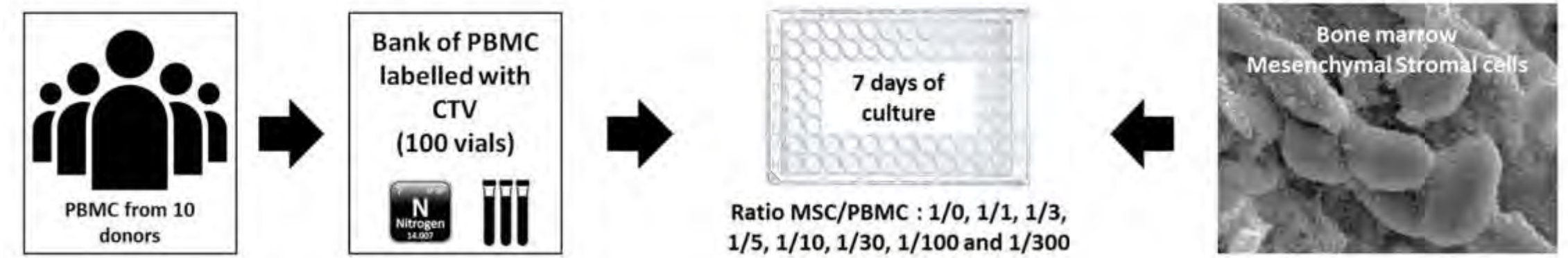
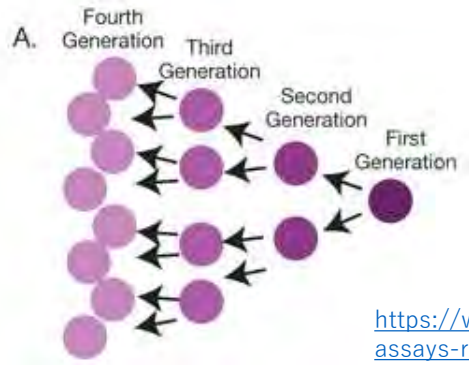
Mixed Lymphocyte Reaction Assay for Mesenchymal Stem/Stromal Cells (MSCs)

Nicotra T, *et al., Stem Cell Res Ther.* 2020;**11:**426.

A. PBMC and MSC co-culture



CellTrace™ Violet (CTV)



Assay Protocol of the Experimental and Clinical Cell Therapy Institute of Salzburg in Austria

https://www.thermofisher.com/jp/ja/home/life-science/cell-analysis/flow-cytometry/flow-cytometry-assays-reagents/cell-proliferation-flow-cytometry/improved-cfse-alternatives-cell-proliferation.html

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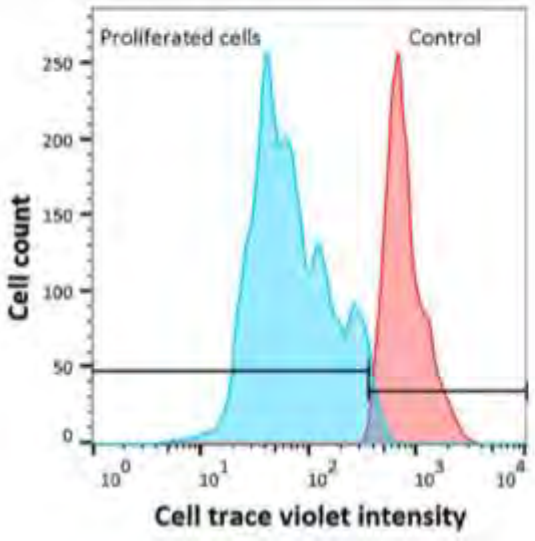
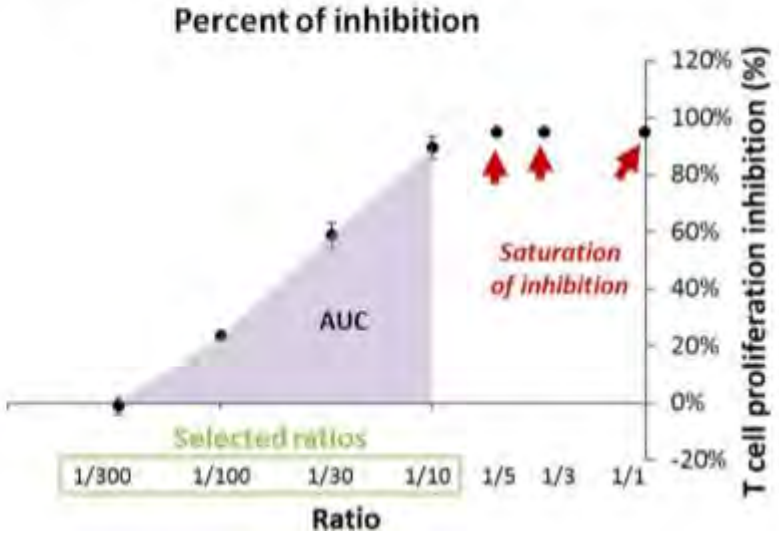
B. Flow cytometry analysis and interpretation





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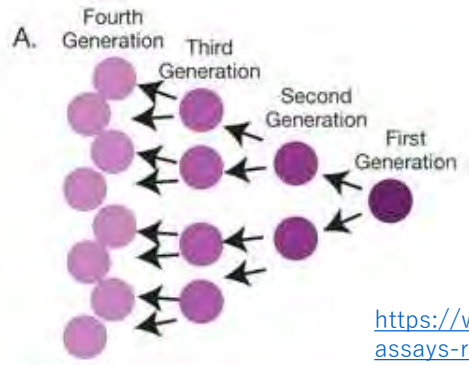

https://www.thermofisher.com/jp/ja/home/life-science/cell-analysis/flow-cytometry/flow-cytometry-assays-reagents/cell-proliferation-flow-cytometry/improved-cfse-alternatives-cell-proliferation.html

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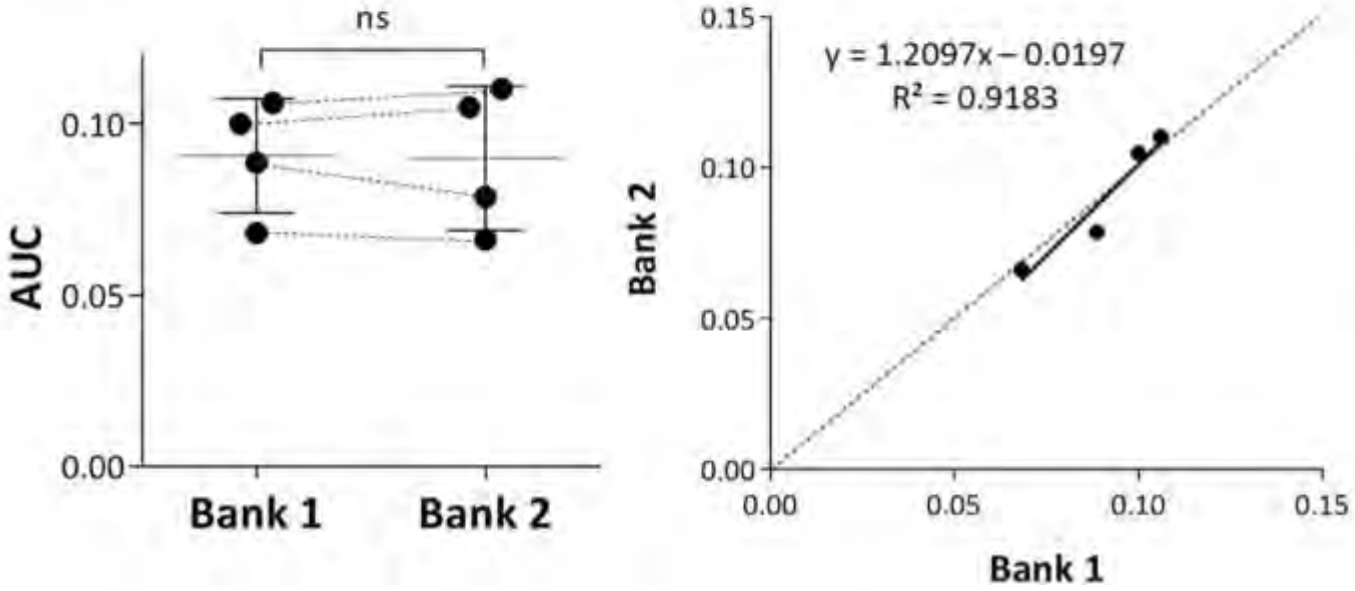
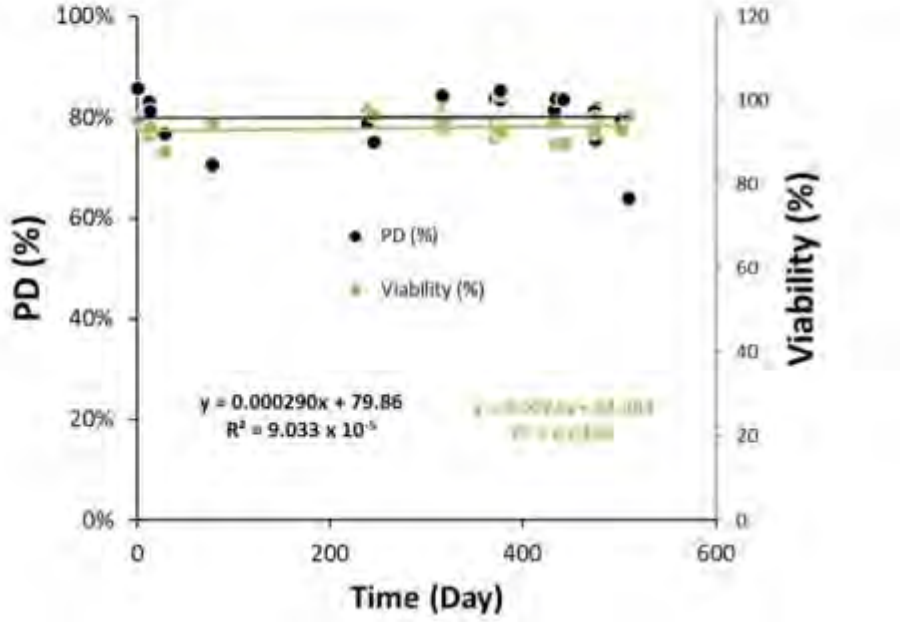
C. Interbank comparison



D. Bank stability



ICH Q2(R1) : VALIDATION OF ANALYTICAL PROCEDURES

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		quantitat.	limit	
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

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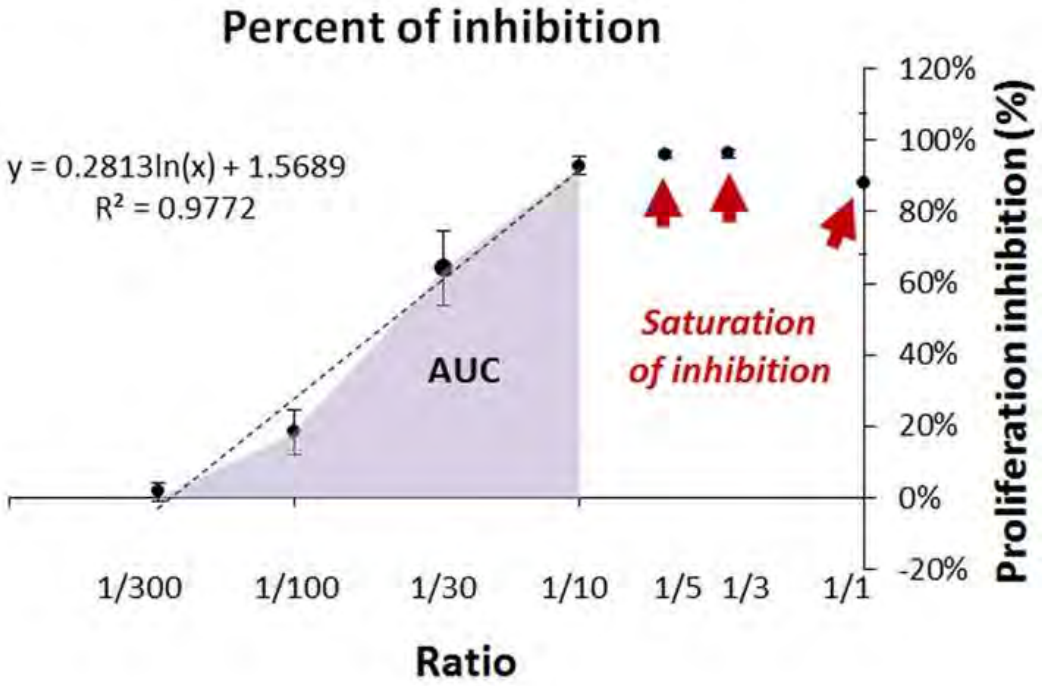
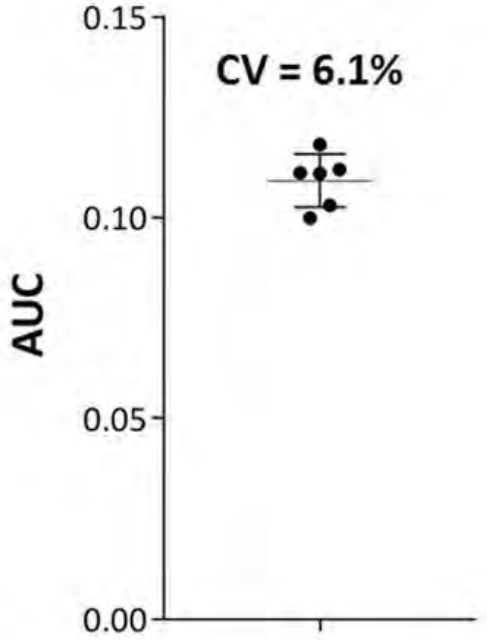
B. Repeatability (n=6)





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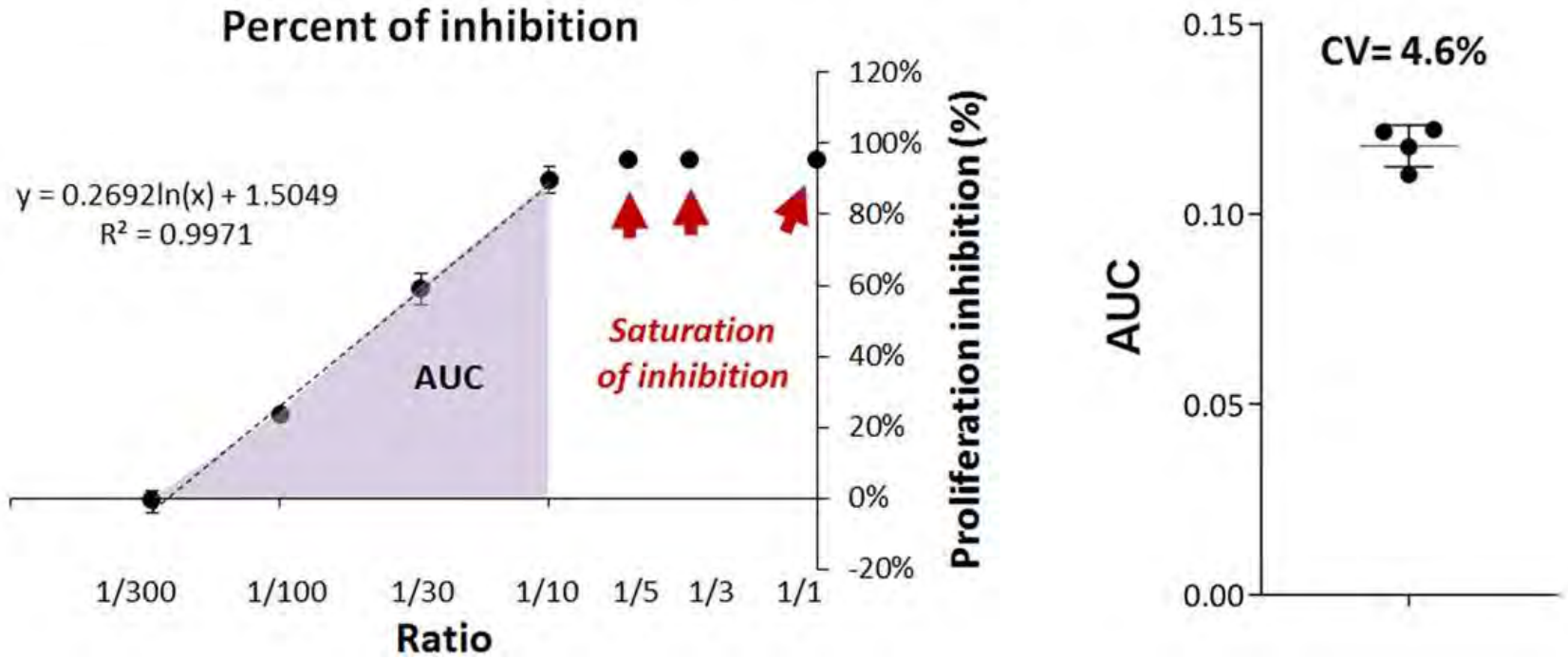
C. Intermediate precision (n=4)



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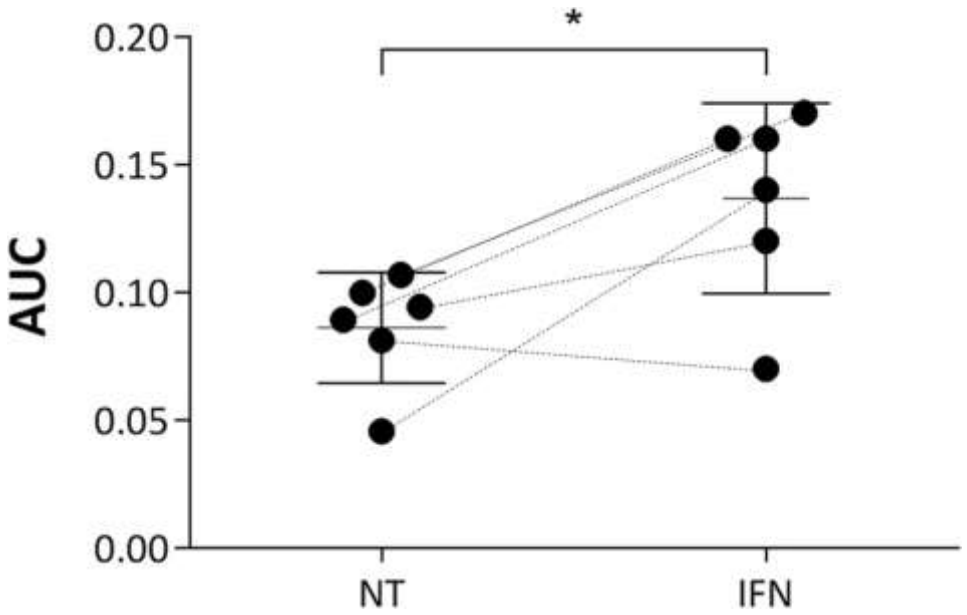
MLR results with MSC primed or not with IFNγ



Conclusions

"The validation data confirm the results of the team of the Experimental and Clinical Cell Therapy Institute of Salzburg in Austria. However, MSC quantification should be checked before culture to reduce variations. Nonetheless, this MLR has sufficiently analytical parameters to quantify the immunomodulatory activity of MSC on T cells. It can be used in the qualification of the pharmaceutical production of MSC batches."

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and

"Validation of Analytical Procedures: Methodology"

except where there are specific issues for unique tests used for analyzing biotechnological and biological products.

Reference standard difficult to obtain

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan

Regenerative Medical Products (CTPs/GTPs) Approved in Japan (as of January 2022)

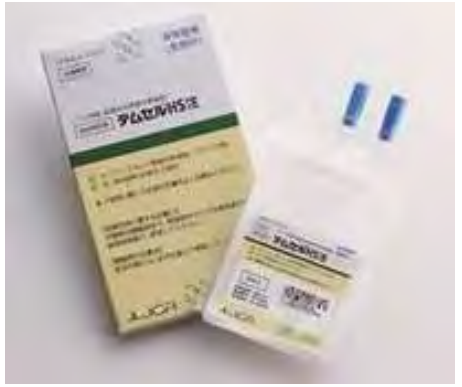
Developer in Japan	Product	Target Disease	Investigator	Remarks
Japan Tissue Engineering Co., Ltd.	JACE (autologous cultured epidermis)	Severe burn *1, Congenital Giant pigmented nevus*2, Epidermolysis bullosa *3	Prof. Ishikawa, Omori Hosp., Toho Univ. Med. Center	*1 Approved on Oct. 29, 2007 *2 Approved on Sep. 29, 2016 *3 Approved on Dec. 28, 2018
Japan Tissue Engineering Co., Ltd.	JACC (autologous cultured cartilage)	Knee cartilage injury		Approved on Jul. 27, 2012
JCR Pharmatheuticals Co., Ltd.	TEMCELL (allogenic mesenchymal stem cell)	Acute GVHD		Approved on Sep. 18, 2015
Terumo Corp.	Heart Sheet (autologous skeletal myoblast sheet)	Serious ischemic heart failure	Prof. Sawa, Osaka Univ.	Approved on Sep. 18, 2015 (Conditional)
Nipro Corp.	STEMIRAC (autologous mesenchymal stem cell)	Spinal cord injury	Prof. Honmou and Prof. Yamashita, Sapporo Med. Univ.	Approved on Dec. 28, 2018 (Conditional) SAKIGAKE designated*
Novartis Pharma K.K.	KYMRIAH (chimeric antigen receptor T cell (CAR-T) therapy)	B-cell acute lymphoblastic leukemia		Approved on Mar. 26, 2019
AnGes, Inc.	Collategene (HGF plasmid product)	Chronic arterial obstruction	Prof. Morishita, Osaka Univ.	Approved on Mar. 26, 2019 (Conditional)
Novartis Pharma K.K.	Zolgensma (AAV vector product)	Spinal muscular atrophy (SMA)		Approved on Mar. 19, 2020
Japan Tissue Engineering Co., Ltd.	Nepic (autologous cultured cornea epithelium)	Corneal epithelial stem cell deficiency	Prof. Nishida, Osaka Univ.	Approved on Mar. 19, 2020
Daiichi Sankyo Co., Ltd.	YESCARTA (chimeric antigen receptor T cell (CAR-T) therapy)	Relapsed or refractory diffuse large B-cell lymphoma, etc.		Approved on Jan. 22, 2021
Celgene K.K.	Breyanzi (chimeric antigen receptor T cell (CAR-T) therapy)	Relapsed or refractory large B-cell lymphoma and relapsed or refractory follicular lymphoma		Approved on Mar. 22, 2021
Japan Tissue Engineering Co., Ltd.	Ocural (autologous cultured oral mucosal epithelium)	Corneal epithelial stem cell deficiency	Prof. Nishida, Osaka Univ.	Approved on Jun. 11, 2021
Daiichi Sankyo Co., Ltd.	DELYTACT Genetically modified herpes virus/G47Δ)	Malignant brain tumor (glial tumor)	Proof. Todo, Univ. of Tokyo	Approved on Jun. 11, 2021 (Conditional) SAKIGAKE designated*
Takeda Pharmaceutical Co., Ltd.	Alofisel (human allogeneic adipose-derived mesenchymal stem cells)	complex perianal fistulas in patients with non-active or mildly active luminal Crohn's disease		Approved on Sep. 27, 2021

* SAKIGAKE (forerunner/vanguard) ≃ Breakthrough Therapy Designation in the U.S.

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan

CASE 1 TEMCELL HS Injection (JCR Pharmatheuticals Co., Ltd.)

Human (allogeneic) bone marrow-derived mesenchymal stem cells



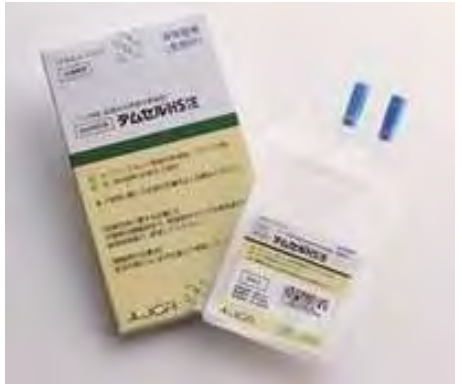
https://mf-p.jp/news_matehan/428.html

- **TEMCELL HS Injection** is **a human (allogeneic) bone marrow-derived mesenchymal stem cell product** obtained by expanded culture of nucleated cells isolated from healthy adult bone marrow fluid.

- It is administered intravenously **to treat acute graft-versus-host disease (acute GVHD)** after hematopoietic stem cell transplantation.

Review Report of TEMCELL (https://www.pmda.go.jp/regenerative_medicines/2015/R20151009001/530210000_22700FZX00001_A100_4.pdf [in Japanese]

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan



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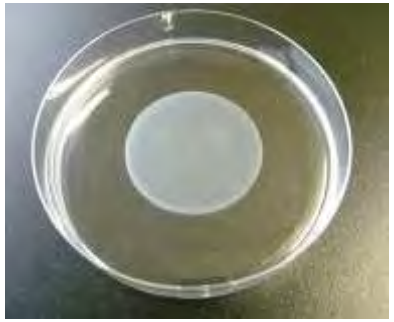
CASE 1 TEMCELL HS Injection (JCR Pharmatheuticals Co., Ltd.)

Characterization of the cellular ingredient

- Immunomodulatory effects
  - Inhibition of human T cell proliferation
  - Effects of PGE_2 synthesis inhibitors and IDO inhibitors on the inhibition of human T cell proliferation
  - Expression of TLR family genes
- Cell migration capacity
  - Expression of cell migration-related genes
  - In vitro cell migration assay
- Cytokine release

Review Report of TEMCELL (https://www.pmda.go.jp/regenerative_medicines/2015/R20151009001/530210000_22700FZX00001_A100_4.pdf [in Japanese]

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan



https://www.terumo.co.jp/medical/regenerative/heartsheet.html

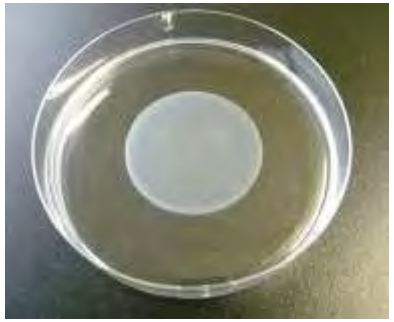
CASE 2 HeartSheet (Terumo Corporation)

Human (autologous) skeletal muscle-derived cell sheet

- **HeartSheet** is a **human (autologous) skeletal muscle-derived cell product** whose main constituent is the patient's own skeletal myoblasts cultured, propagated and cryopreserved.
- This product includes as a subcomponent instruments for cell sheeting in medical institutions.
- By applying the product as a sheet of cells to the surface of the heart through open heart surgery, it is **used to treat severe heart failure caused by ischemic heart disease** that is insufficiently responsive to standard treatment.

Review Report of HeartSheet (https://www.pmda.go.jp/regenerative_medicines/2015/R20151008001/470034000_22700FZX00002_A100_2.pdf [in Japanese]

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan



https://www.terumo.co.jp/medical/regenerative/heartsheet.html

CASE 2 HeartSheet (Terumo Corporation)

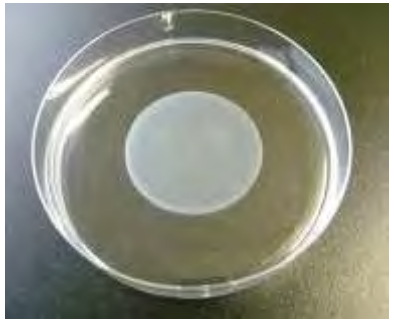
MOA for improving cardiac function

- "In cardiac tissues 13 weeks after transplantation into pigs, no evidence of engraftment or differentiation of porcine cell sheets was observed."
- "An increase in cardiac function in the transplant group was observed from the time of 1 week after transplantation."

- "These results suggest that the effect of skeletal myoblast cell sheet transplantation on cardiac function is not due to long-term engraftment or differentiation of the transplanted cells, but rather to some initial response brought about by the transplanted cells."

Review Report of HeartSheet (https://www.pmda.go.jp/regenerative_medicines/2015/R20151008001/470034000_22700FZX00002_A100_2.pdf [in Japanese]

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan



https://www.terumo.co.jp/medical/regenerative/heartsheet.html

CASE 2 HeartSheet (Terumo Corporation)

Product characterization: quantification of biologically active substances

- "[redacted] kinds of bioactive substances [redacted], which were assumed to be produced by skeletal myoblasts, in [redacted] from the skeletal myoblast sheet preparations were measured by ELISA, and the production of **HGF**, **VEGF**, and **SDF-1** was confirmed."

- "The relationship between the production of these substances and the efficacy and safety of the product has not been clarified, and will be assessed by quantifying **VEGF**, **HGF**, and **SDF-1** as a characterization procedure using the collected [redacted] from medical institutions."

Review Report of HeartSheet (https://www.pmda.go.jp/regenerative_medicines/2015/R20151008001/470034000_22700FZX00002_A100_2.pdf [in Japanese]

Analytical Techniques → Product Characterization → Specifications

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]

Analytical Techniques for determination of

Physicochemical Properties, **Biological Activity**, Immunochemical Properties, Purity & Impurities

↓

Product Characterization → **Extensive characterization needs to be performed**

1) **in the development phase and,**

2) **where necessary, following significant process changes.**

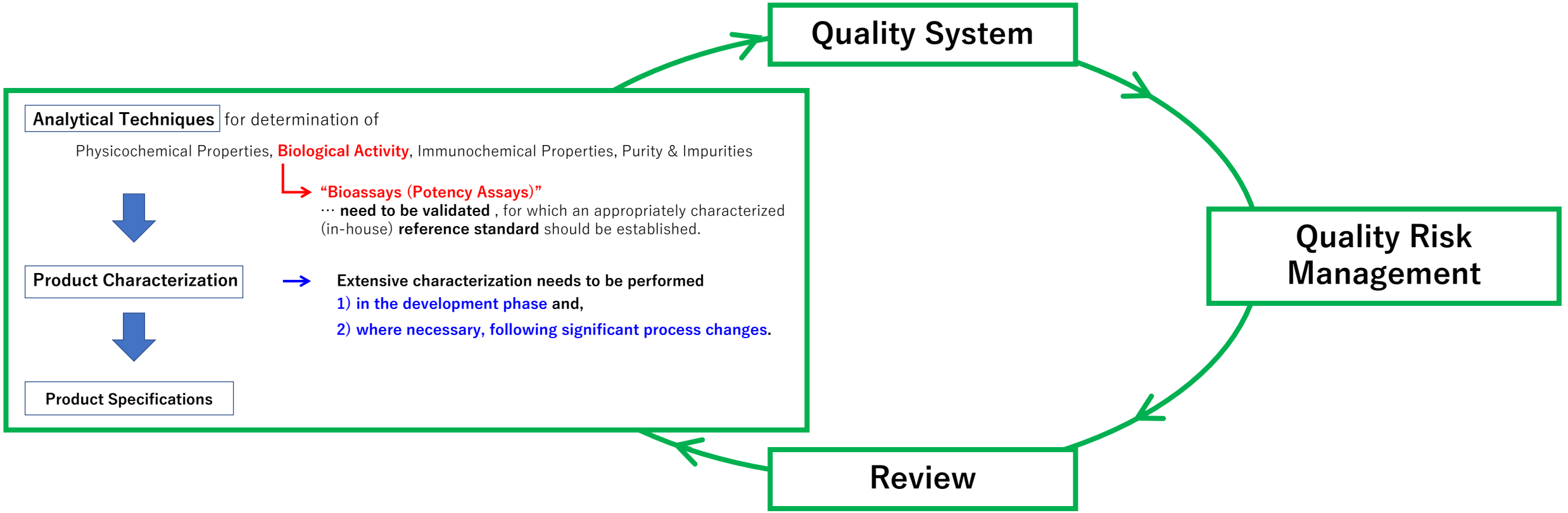
↓

Product Specifications

"Bioassays (Potency Assays)"
… **need to be validated** , for which an appropriately characterized (in-house) **reference standard** should be established.

Analytical Techniques → Product Characterization → Specifications

ICH Q6B [CTPs are outside the scope, but the principles may apply to CTPs]



Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan

https://www.nikkei.com/article/DGXZQOUC019GZ0R01C21A1000000/

CASE 3 DELYTACT (Daiichi Sankyo Co., Ltd.)

Oncolytic recombinant herpes simplex virus type 1

- **DELYTACT** is **a recombinant herpes simplex virus type 1** in which the α47 and γ34.5 genes have been deleted and the ICP6 gene has been inactivated by insertion of the E. coli-derived lacZ gene.

- It is used for the treatment of patients with malignant glioma.

Review Report of DELYTACT (https://www.pmda.go.jp/regenerative_medicines/2021/R20210629001/430574000_30300FZX00004_A100_1.pdf [in Japanese]

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan

https://www.nikkei.com/article/DGXZQOUC019GZ0R01C21A1000000/

CASE 3 DELYTACT (Daiichi Sankyo Co., Ltd.)

Product characterization: biological activity

- “A titer-dependent [redacted] effect was observed against human [redacted] cell line [redacted]. As a test item for [redacted] activity, potency is set in the specifications and test methods, and [redacted] is [redacted] of [redacted].

Review Report of DELYTACT (https://www.pmda.go.jp/regenerative_medicines/2021/R20210629001/430574000_30300FZX00004_A100_1.pdf [in Japanese]

Case studies on potency assays (bioassays) in product characterizations of CTPs approved in Japan

https://www.nikkei.com/article/DGXZQOUC019GZ0R01C21A1000000/

CASE 3 DELYTACT (Daiichi Sankyo Co., Ltd.)

Tests to support efficacy or performance

- *In vitro* cytotoxic activity test
  - The cytotoxic activity of this product against human [redacted] cell line [redacted] was correlated with MOI.

- Acyclovir sensitivity test
  - [redacted] cells were used to evaluate the sensitivity of the product to acyclovir, and it was observed that [redacted] of G47Δ was decreased in a concentration-dependent manner.

Review Report of DELYTACT (https://www.pmda.go.jp/regenerative_medicines/2021/R20210629001/430574000_30300FZX00004_A100_1.pdf [in Japanese]

In Summary …

- Sponsors should begin efforts to measure potency early in the development of CTPs. Understanding their mechanisms of action is essential for establishing and selecting potency assays (bioassays).
- Potency assays (bioassays) should be evolved/improved throughout development, from early to late development.
- Potency assays (bioassays) for CTPs tend to be product-specific or class-specific, not one-size-fits-all. The assays need to be validated according to ICH Q2(R1), but there are some limitations due to the unavailability of well-established reference standards, *etc.*
- It is likely that a single assay will not provide sufficient information to predict the efficacy of a CTP, and it is anticipated that a matrix of potency assays (bioassays) will often be required for characterization and release specifications of CTPs.

Thank you for your attention!

Yoji SATO, PhD

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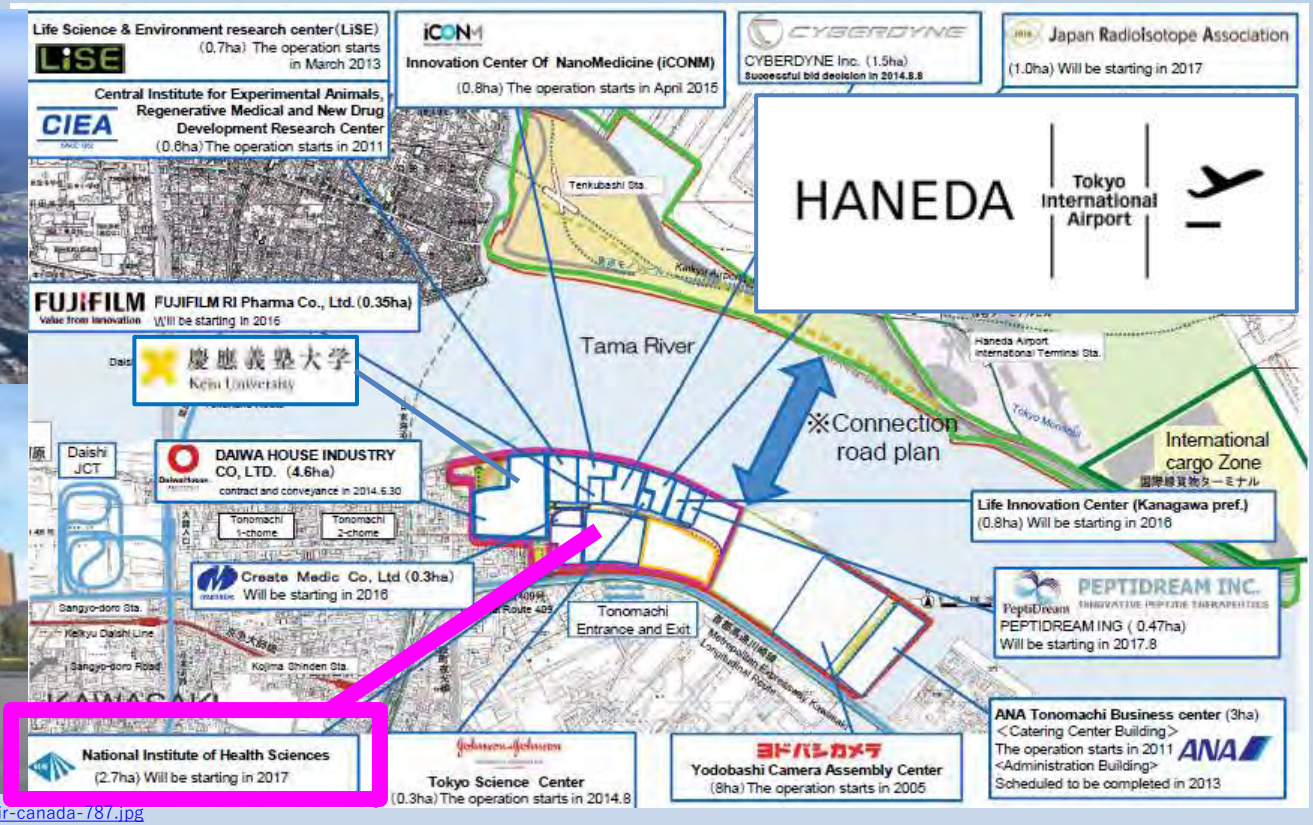

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* https://www.oag.com/hubfs/air-canada-787.jpg
** http://www.city.kawasaki.jp/en/page/0000038680.html

Supplemental Information

GLOSSARY (1)
ICH Q2(R1) : VALIDATION OF ANALYTICAL PROCEDURES

- **ACCURACY:** The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

- **PRECISION:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

GLOSSARY (2)
ICH Q2(R1) : VALIDATION OF ANALYTICAL PROCEDURES

- **REPEATABILITY:** Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision .

- **INTERMEDIATE PRECISION:** Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

- **REPRODUCIBILITY:** Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

GLOSSARY (3)
ICH Q2(R1) : VALIDATION OF ANALYTICAL PROCEDURES

- **SPECIFICITY:** Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

  Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

  This definition has the following implications:

  - Identification: to ensure the identity of an analyte.
  - Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.
  - Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

GLOSSARY (4)
ICH Q2(R1) : VALIDATION OF ANALYTICAL PROCEDURES

- **DETECTION LIMIT:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

- **QUANTITATION LIMIT:** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

GLOSSARY (5)
ICH Q2(R1) : VALIDATION OF ANALYTICAL PROCEDURES

- **LINEARITY:** The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

- **RANGE:** The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

- **ROBUSTNESS:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.