The 24th JSRM Congress
March 22, 2025, Yokohama

シンポジウム26　再生細胞医療を日本の基幹産業に育てるために必要な「公（おおやけ）の理念」

Ensuring Developer Access to Quality Testing Methods and Related Information for Cell Therapy Products

細胞加工製品の品質評価技術や関連情報へのアクセスの確保

Yoji SATO, Ph.D.

Head, Division of Drugs, National Institute of Health Sciences

Visiting Professor, School of Health Innovation, Kanagawa University of Human Services

Adjunct Research Scientist, Kanagawa Institute of Industrial Science and Technology

DISCLAMER

細胞加工製品の品質評価技術や関連情報へのアクセスの確保

佐藤陽治
国立医薬品食品衛生研究所
薬品部

筆頭演者は、過去１年間（1月～12z月）において、本演題の発表に関して開示すべきCOIはありません。

本発表で述べられた見解・意見は発表者のものであり、必ずしも国立医薬品食品衛生研究所または厚生労働省の公式な方針や見解を示すものではありません。

Ensuring Developer Access to Quality Testing Methods and Related Information for Cell-Processed Products

Yoji Sato, Ph.D.,
Division of Drugs,
National Institute of Health Sciences

For the past year (January-December), the speaker has no COI to disclose for this presentation

The views and opinions expressed in this presentation are those of the presenter and do not necessarily represent official policy or position of the National Institute of Health Sciences, or the Ministry of Health, Labour & Welfare.

Challenges for the Industrialization of Cell Therapy Products (CTPs)

主成分である**細胞の品質特性**を知らないと、何を監視・管理すべきか分からない！

- 有効性と安全性
- 有効性と安全性の確保のための品質のあり方
- **品質を確保するための 規格設定・特性解析**
- 適切な規格・試験法にもとづく製造工程設計・機械化・自動化
- 柔軟かつ確固とした製造工程・機械化・自動化による生産性向上
- 生産性向上による製品の収益向上・流通の持続可能性確保

Without knowing the attributes of the cells as the major ingredient, it is impossible to know what should be monitored and controlled for the Q/E/S!

- Efficacy and Safety of CTPs
- Quality for ensuring their Efficacy and Safety
- **Specifications and Test Procedures for their Quality Attributes**
- Manufacturing Process Design, Mechanization and Automation based on their Specifications and Test Procedures
- Improved productivity through flexible and robust processes, mechanization, and automation of cell manufacturing
- Improving their profitability and ensuring the sustainability of the product distribution by improving their productivity

Unique Features of Cell Therapy Products

Variability in quality between lots of a finished product and between lots of its starting material/intermediate product
最終製品のロット間や原料／中間製品のロット間の品質のばらつき

Variability in traits between individual cells in a finished product and its starting material/intermediate product
最終製品中の個々の細胞の間や原料／中間製品中の個々の細胞の間の形質のばらつき

"Heterogeniety"
of the Product and its Raw Materials

製品／原料の「不均質性」

"Inhomogeniety"
of Cell Populations

細胞集団の「不均一性」

Challenges for the Industrialization of Cell Therapy Products (CTPs)

主成分である**細胞の品質特性**を知らないと、**柔軟で確固とした製造工程**は作れない！

- **有効性**と**安全性**
- **有効性**と**安全性**の確保のための**品質**のあり方
- **品質**を確保するための **規格設定・特性解析**
- **適切な規格・試験法**にもとづく**製造工程設計・機械化・自動化**
- **柔軟かつ確固とした製造工程・機械化・自動化**による**生産性向上**
- **生産性向上**による**製品の収益向上・流通の持続可能性確保**

Without knowing the attributes of the cells as the major ingredient, it is impossible to achieve flexible and robust manufacturing processes!

- Efficacy and Safety of CTPs
- Quality for ensuring their Efficacy and Safety
- Specifications and Test Procedures for their Quality Attributes
- Manufacturing Process Design, Mechanization and Automation based on their Specifications and Test Procedures
- Improved productivity through flexible and robust processes, mechanization, and automation of cell manufacturing
- Improving their profitability and ensuring the sustainability of the product distribution by improving their productivity

Essential Requirement for Changes in the Manufacturing Process of Biological Products, including CTPs
細胞加工製品を含むバイオ医薬品等の製造工程の変更時の必須要件

- The changes in the manufacturing process should **not adversely affect the product safety and efficacy**.
  - It is reasonable and effective to judge the pros and cons of changing the manufacturing method by **evaluating changes in the quality attributes of the product before and after the change**.
  - The need for confirmation in non-clinical and clinical trials is also determined by the content of the quality attribute evaluation.

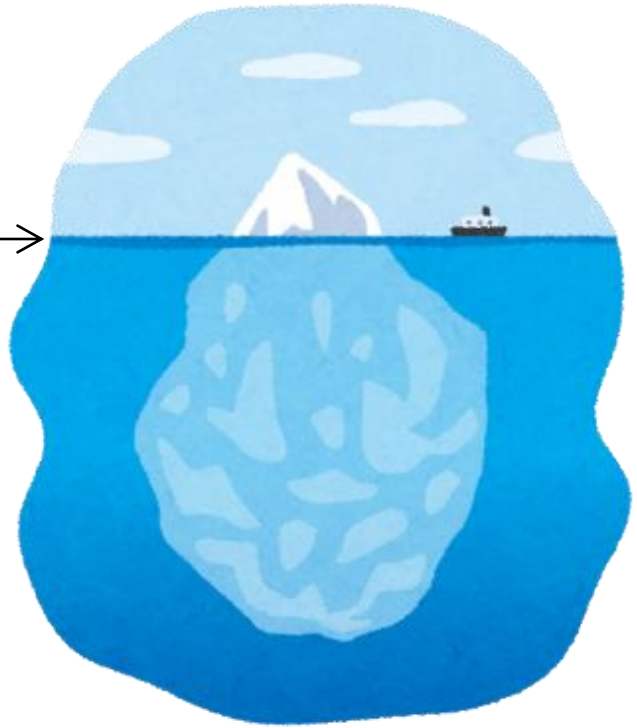
- 製法変更によって少なくとも**製品の安全性と有効性に有害な影響を及ぼす変化がない**こと
  - 製法変更の是非は、変更前後の製品の**品質特性の変化を評価**することにより判断することが合理的かつ効果的。
  - 非臨床試験・臨床試験による確認尾必要性も、品質特性の評価の内容次第で判断。

Cell Therapy Products are Complex
細胞加工製品は複雑

Limited Characterization Window
(Recognizable Quality Attributes)

Limit of Knowledge

Hidden/Unrecognizable
(but Potentially Critical)
Quality Attributes



Efficacy

Safety

…which creates UNCERTAINTY in the comparability assessment
(観察可能な)品質特性データのみで同等性を評価・保証することは難しいと予想される

Challenges in Exploring and Evaluating Critical Quality Attributes (CQAs)

重要品質特性（CQA）を探索・評価する際の課題

Test methods for viral safety, sterility, and **tumorigenicity**

- **Safety-related CQAs (characteristics and quantity of hazards)**

  Can you detect hazards and hazardous impurities that may have proliferative potential?

  Do you understand the sensitivity of your assays?

  = How can you avoid false negatives (and false positives)?

- **Efficacy-related CQAs**

  How do you identify attributes linked to cellular functions that

  … It's very difficult for products with unclear mechanis

ウイルス安全性や無菌性**造腫瘍性**の評価方法

- **安全性関連のCQA（ハザードの質と量）**

  増殖能を示すハザード・有害不純物を漏れなく検出できているか？測定法の感度を理解しているか？

  =偽陰性（＆偽陽性）の回避

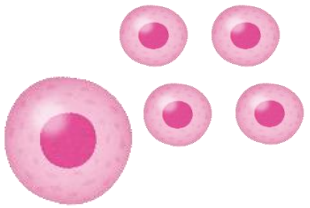
- **有効性関連のCQA**

  有効性を裏付ける細胞機能とリンクした細胞特性をいかに同定する（掘り当てる）か？

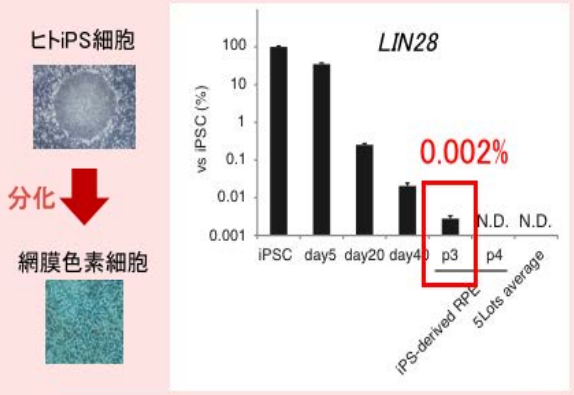
  ・・・ 作用機序が明確でない製品の場合は、とても難しい

Development and Validation of Highly Sensitive Detection Method for Tumorigenic Cells Intermingled in CTPs

Detection of residual ES/iPS cells

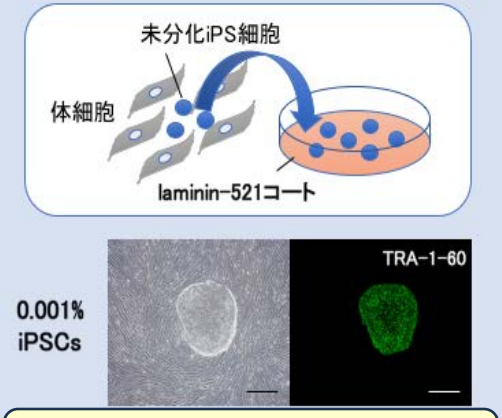


qRT-PCRs



LOD: 0.02% iPSC in RPE cells

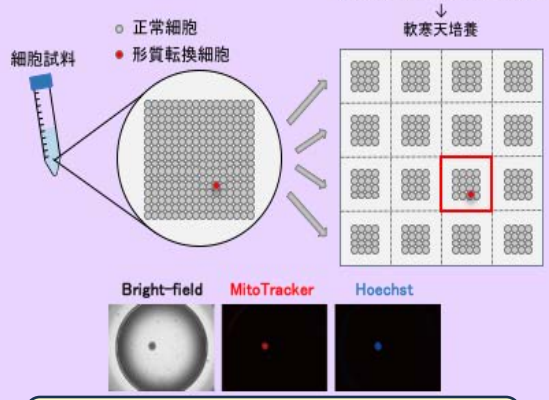
Highly Efficient Culture Assay



LOD: 0.001% iPSC in MSCs

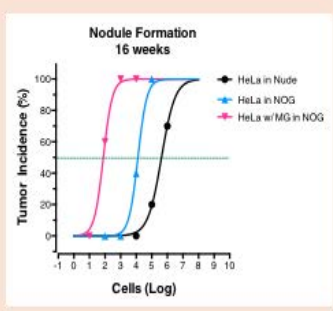
Detection of malignant transformed cells

Digital Soft Agar Colony Formation Assay



LOD: 0.0001% HeLa cells in MSCs

In Vivo Tumorigenicity Test



	TPD50	Fold
Nude	4.0×10^5	1
NOG	1.3×10^4	25
NOG+ Matrigel	7.9×10	5,000

5,000-fold more sensitive compared with the conventional protocol

MHLW Guidance Document

“Points to consider for the detection of undifferentiated pluripotent stem cells and transformed cells, tumorigenicity testing, and genetic stability evaluation of human-derived cell processed products”

目次

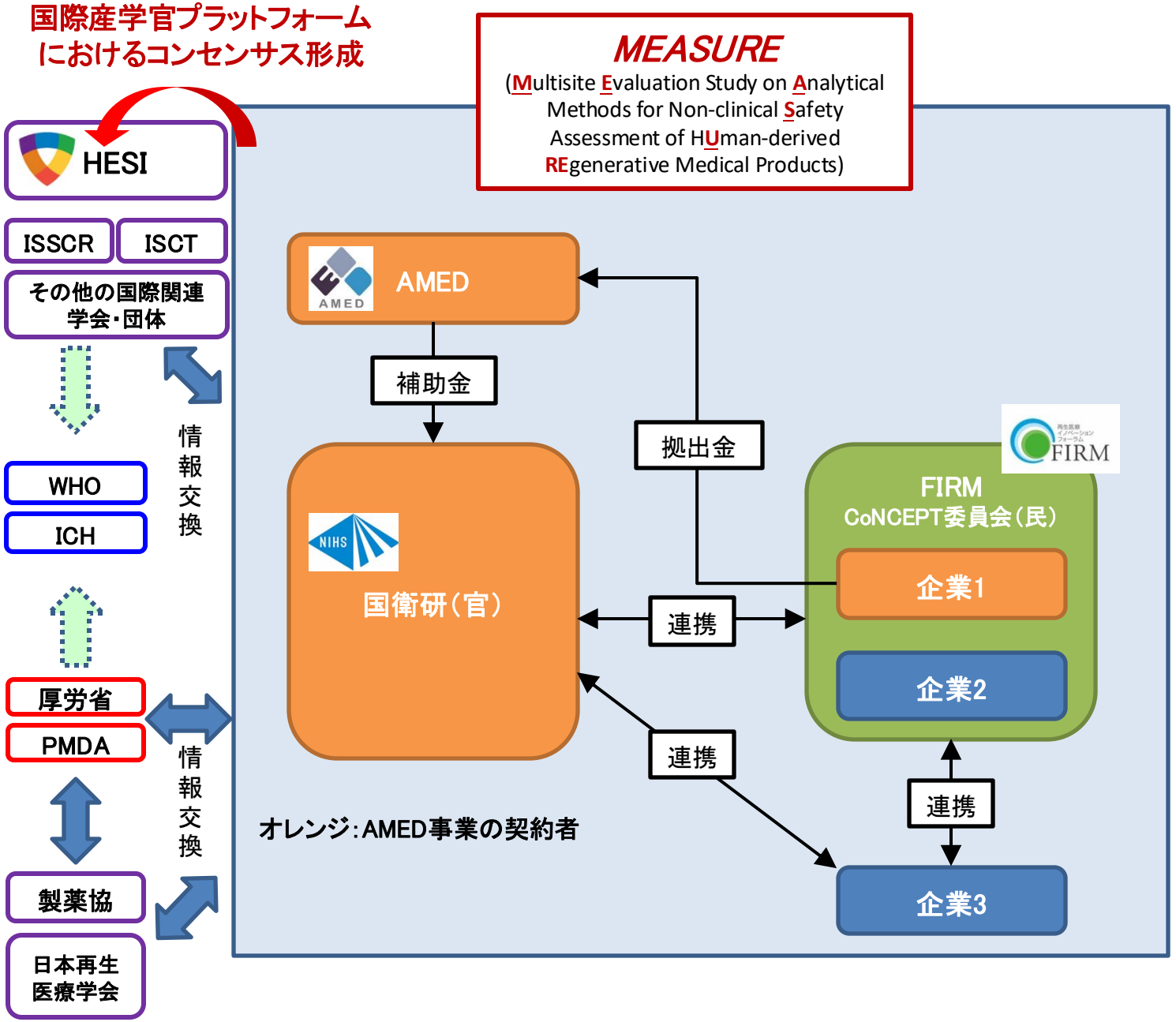
Notification 0627-1 issued on 27 June 2019 by Director, Office of Medical Devices Evaluation, Pharmaceuticals and Food Safety Bureau, MHLW
厚生労働省 薬生機審発0627第1号通知，令和元年6月27日

厚生労働省
Ministry of Health, Labour and Welfar

Assay Protocols

NIHS

国際産学官プラットフォーム
におけるコンセンサス形成



企業1
試験統括機関

富士フイルム株式会社
住友ファーマ株式会社
武田薬品工業株式会社
テルモ株式会社
アステラス製薬株式会社
協和キリン株式会社*

*：2019年度まで

企業2
FIRM会員の
研究協力者および
研究補助者

企業3
FIRM会員以外の
研究協力者および
研究補助者

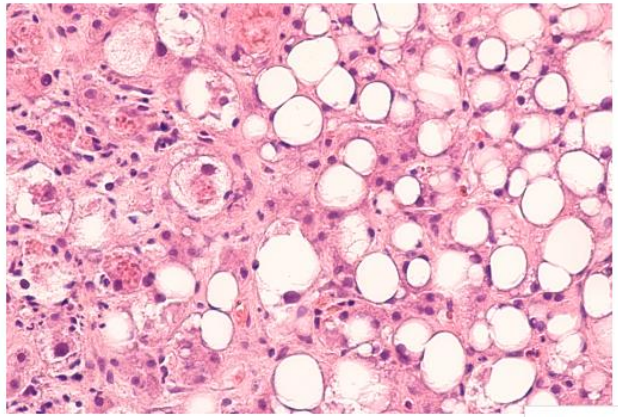
INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

国際幹細胞学会タスクフォースによる
多能性幹細胞加工製品開発のベスト・プラクティス文書

Best Practices for the Development of PSC-Derived Cellular Therapies

日本の規制・評価手法の国際文書への導入（⇒製品の国際開発促進）
近日公開予定

Currently underway, this initiative will provide recommendations to facilitate and streamline the development of PSC-based cellular therapies regardless of regulatory jurisdiction. It will also provide detailed guidance at key product development pain points.



English

Challenges in Exploring and Evaluating Critical Quality Attributes (CQAs)

重要品質特性（CQA）を探索・評価する際の課題

- **Safety-related CQAs (characteristics and quantity of hazards)**

  Can you detect hazards and hazardous impurities that may have proliferative potential?

  Do you understand the sensitivity of your assays?

  = How can you avoid false negatives (and false positives)?

- **Efficacy-related CQAs**

  How do you identify attributes linked to cellular functions that support efficacy?

  … It's very difficult for products with unclear mechanisms of action.

- **安全性関連のCQA（ハザードの質と量）**

  増殖能を示すハザード・有害不純物を漏れなく検出できているか？測定法の感度を理解しているか？

  =偽陰性（＆偽陽性）の回避

- **有効性関連のCQA**

  有効性を裏付ける細胞機能とリンクした細胞特性をいかに同定する（掘り当てる）か？

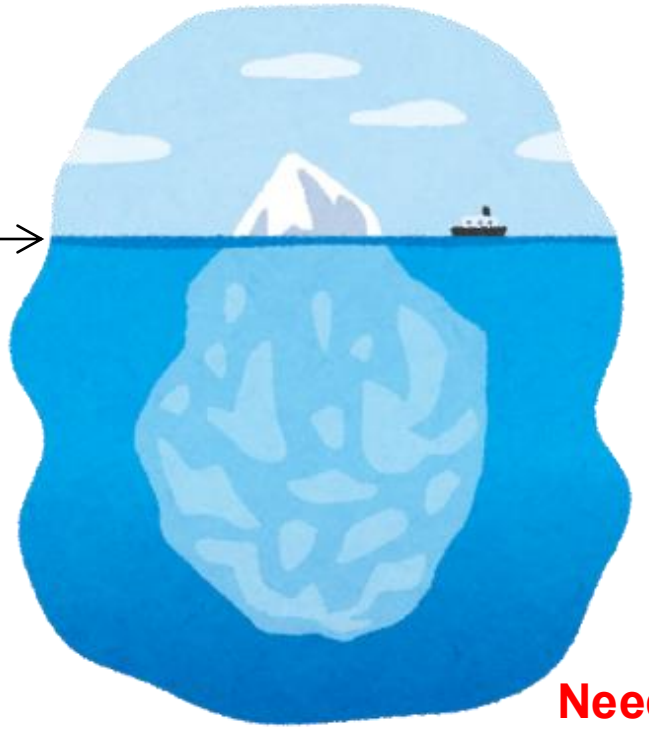
  ・・・ 作用機序が明確でない製品の場合は、とても難しい

Cell Therapy Products are Complex
細胞加工製品は複雑

Limited Characterization Window
(Recognizable Quality Attributes)

Limit of Knowledge

Hidden/Unrecognizable
(but Potentially Critical)
Quality Attributes



Efficacy

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

Need for understanding MOA and CQAs related to the efficacy or *in vitro* potency.

Need for a tool for uncovering hidden CQAs

Need for Technology to Understand Heterogeneity/ Inhomogeneity
不均質性／不均一性を理解するための技術が必要

For example, **even when there are a total of 1 million cells, only 10,000 of them may be effective**.

"Visualization" of such inhomogeneity and **characterization of those 10,000 cells** would **make identifying CQAs related to efficacy easier.**

例えば、**総細胞数が100万個**あっても、**そのうち有効性を発揮するのは1万個**しかないという場合もありうる。

このような**不均一性を「見える化」**することで、**その1万個の細胞がどのような特性を持つのか**を明らかにすれば、 **有効性に関連するCQA（重要品質特性）を発見しやすくなる（・・・と期待できる）**

EXAMPLE (A)

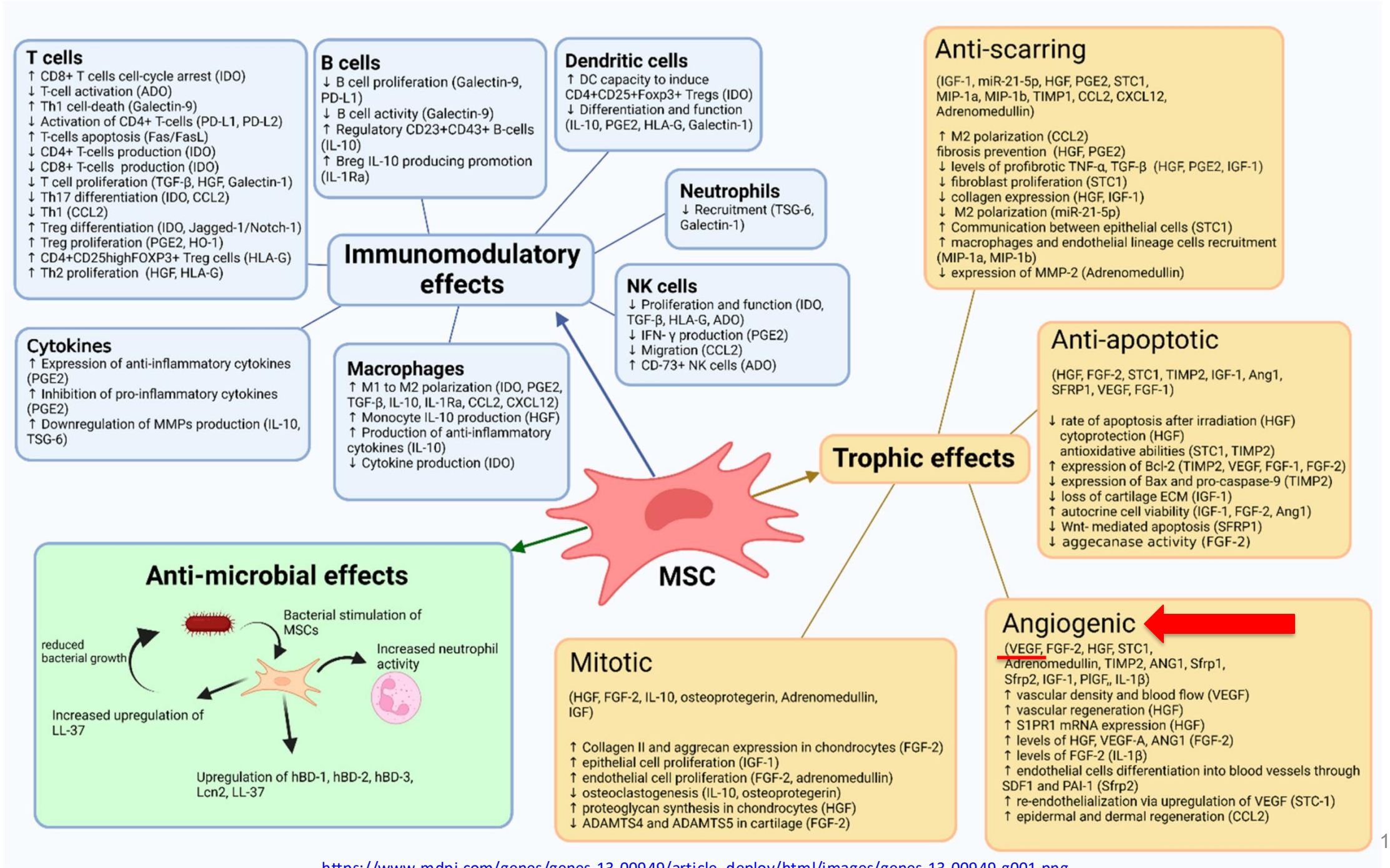
Stem Cells Translational Medicine, 2023, **12**, 379–390
https://doi.org/10.1093/stcltm/szad029
Advance access publication 2 June 2023
Original Research

Single-Cell RNA-Seq Reveals *LRRC75A*-Expressing Cell Population Involved in VEGF Secretion of Multipotent Mesenchymal Stromal/Stem Cells Under Ischemia

Takumi Miura[1,2,‡], Tsukasa Kouno[3,‡], Megumi Takano[1], Takuya Kuroda[1], Yumiko Yamamoto[3], Shinji Kusakawa[1], Masaki Suimye Morioka[3], Tohru Sugawara[2,4], Takamasa Hirai[1], Satoshi Yasuda[1], Rumi Sawada[1], Satoko Matsuyama[1,5], Hideya Kawaji[3,6], Takeya Kasukawa[3], Masayoshi Itoh[3], Akifumi Matsuyama[5], Jay W. Shin[3,7], Akihiro Umezawa[2], Jun Kawai[3,8], Yoji Sato[*,1,8,9]

[1]Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, Kanagawa, Japan
[2]Center for Regenerative Medicine, National Center for Child Health and Development, Tokyo, Japan
[3]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[4]Biopharmaceutical and Regenerative Sciences, Graduate School of Medical Life Science, Yokohama City University, Yokohama, Japan
[5]Center for Reverse TR, Osaka Habikino Medical Center, Osaka Prefectural Hospital Organization, Osaka, Japan
[6]Research Center for Genome & Medical Sciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
[7]Genomic Institute of Singapore, Agency for Science, Technology and Research, Singapore
[8]Life Science Technology Project, Kanagawa Institute of Industrial Science and Technology, Kawasaki, Japan
[9]Department of Cellular and Gene Therapy Products, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

*Corresponding author: Yoji Sato, PhD, Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki Ward, Kawasaki City, Kanagawa 210-9501, Japan. Email: yoji@nihs.go.jp
‡Contributed equally.



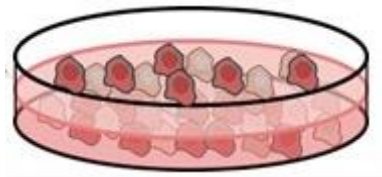
https://www.mdpi.com/genes/genes-13-00949/article_deploy/html/images/genes-13-00949-g001.png

Miura T *et al., Stem Cells Transl Med.* 2023;**12**:379-390.

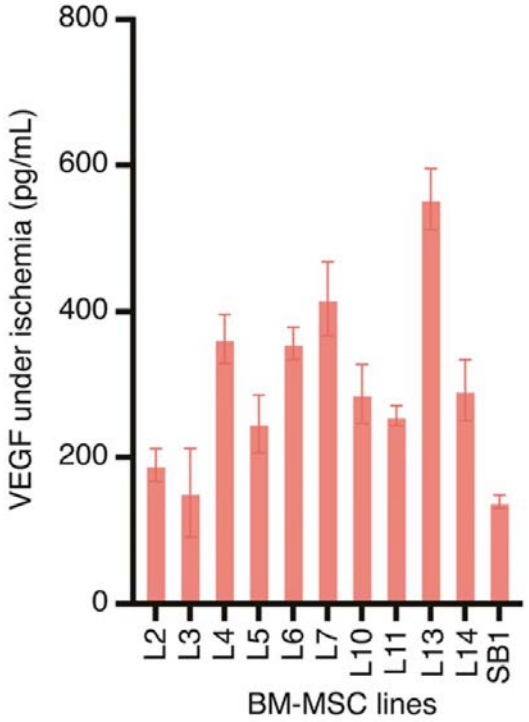
Secretion of angiogenic factors under conditions that mimic the environment (ischemia) at the site of implantation

VEGF secretion under ischemic conditions

hBM-MSCs (PS#5)
Hypoxia
Glucose-free



VEGF
(vascular endothelial growth factor)

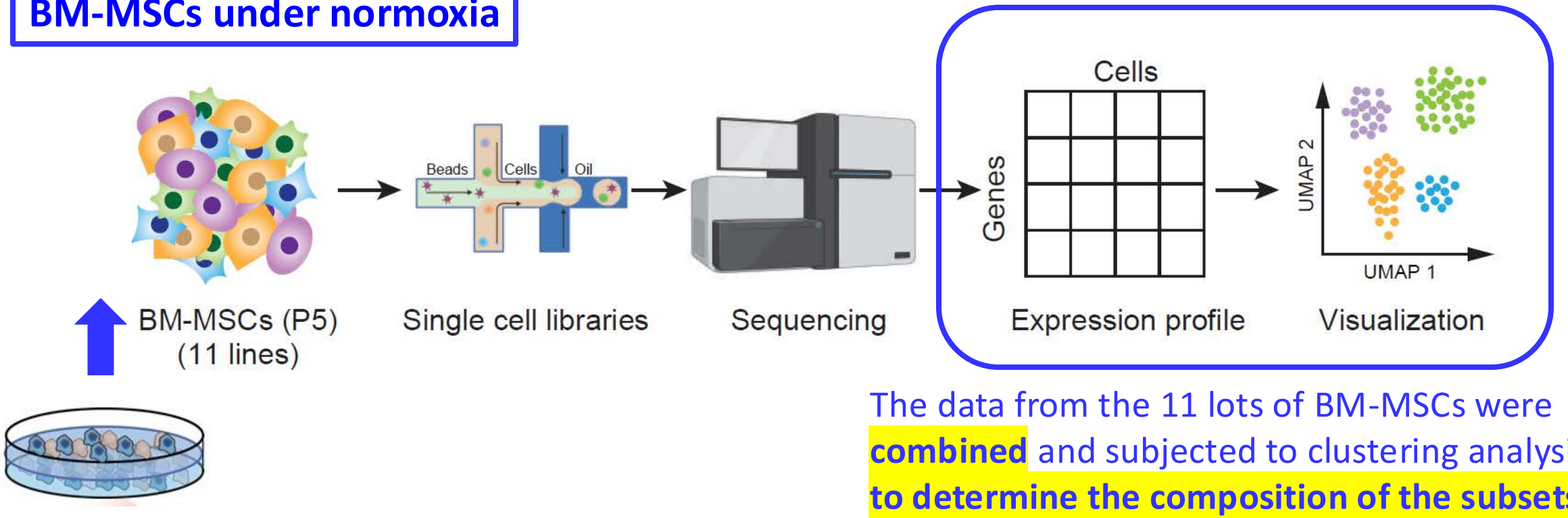


VEGF secretion is highly variable between cell lots.

Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

Single-Cell Transcriptome Experiments

BM-MSCs under normoxia

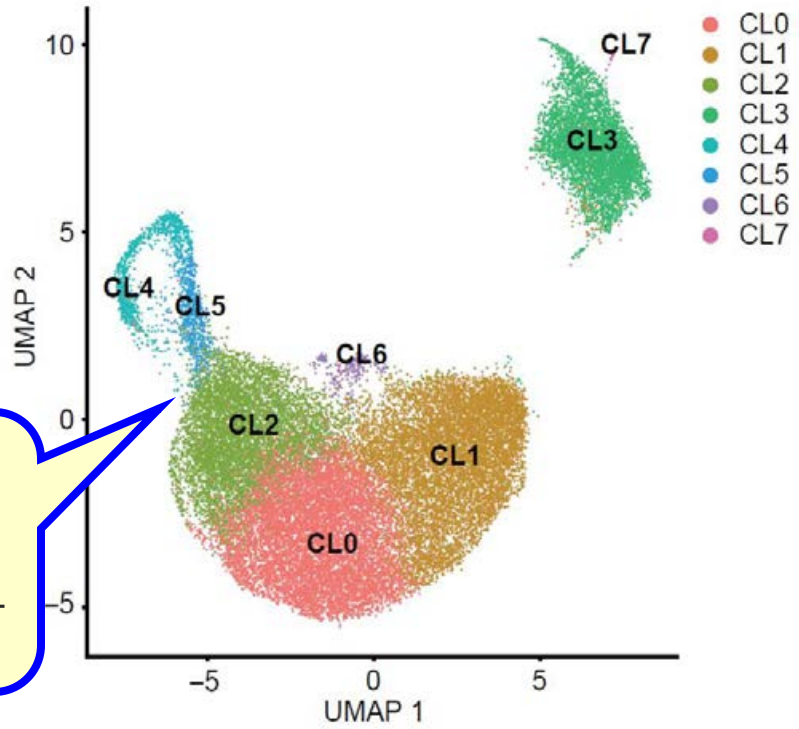


The data from the 11 lots of BM-MSCs were **combined** and subjected to clustering analysis **to determine the composition of the subsets of "average BM-MSCs" (BM-MSCs as a population).**

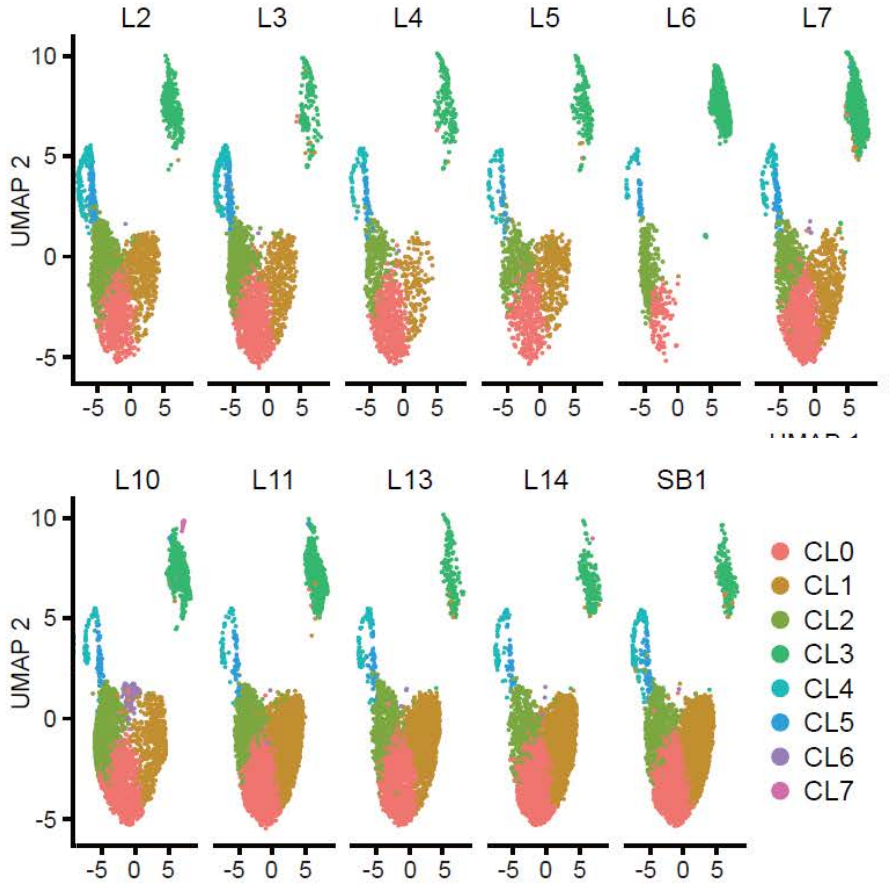
Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

Single-Cell Transcriptome Experiments

BM-MSCs (P5)
Normoxia



Integration and clustering of data from 11 lots of human bone marrow-derived MSCs



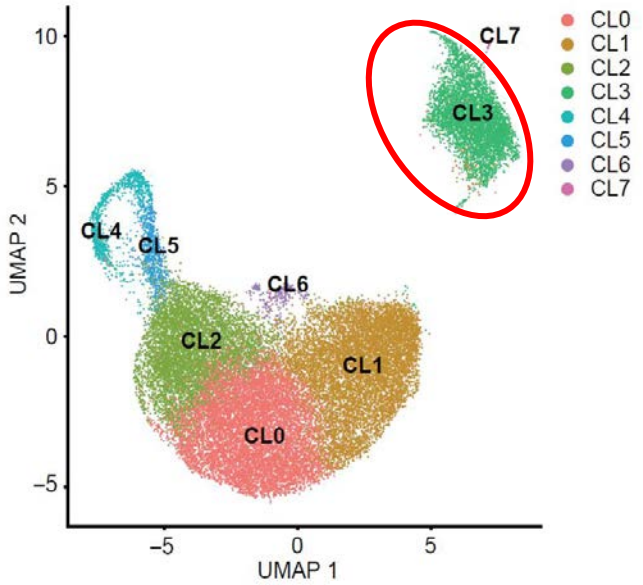
The population of bone marrow-derived MSCs was estimated to consist of 8 clusters

The degree to which the "inhomogeneity of each lot" deviates from the "inhomogeneity of the population" is visible.

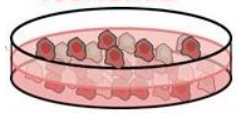
Miura T *et al., Stem Cells Transl Med.* 2023;**12:**379-390.

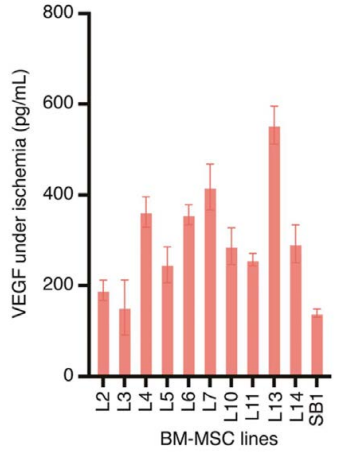
Single-Cell Transcriptome Experiments

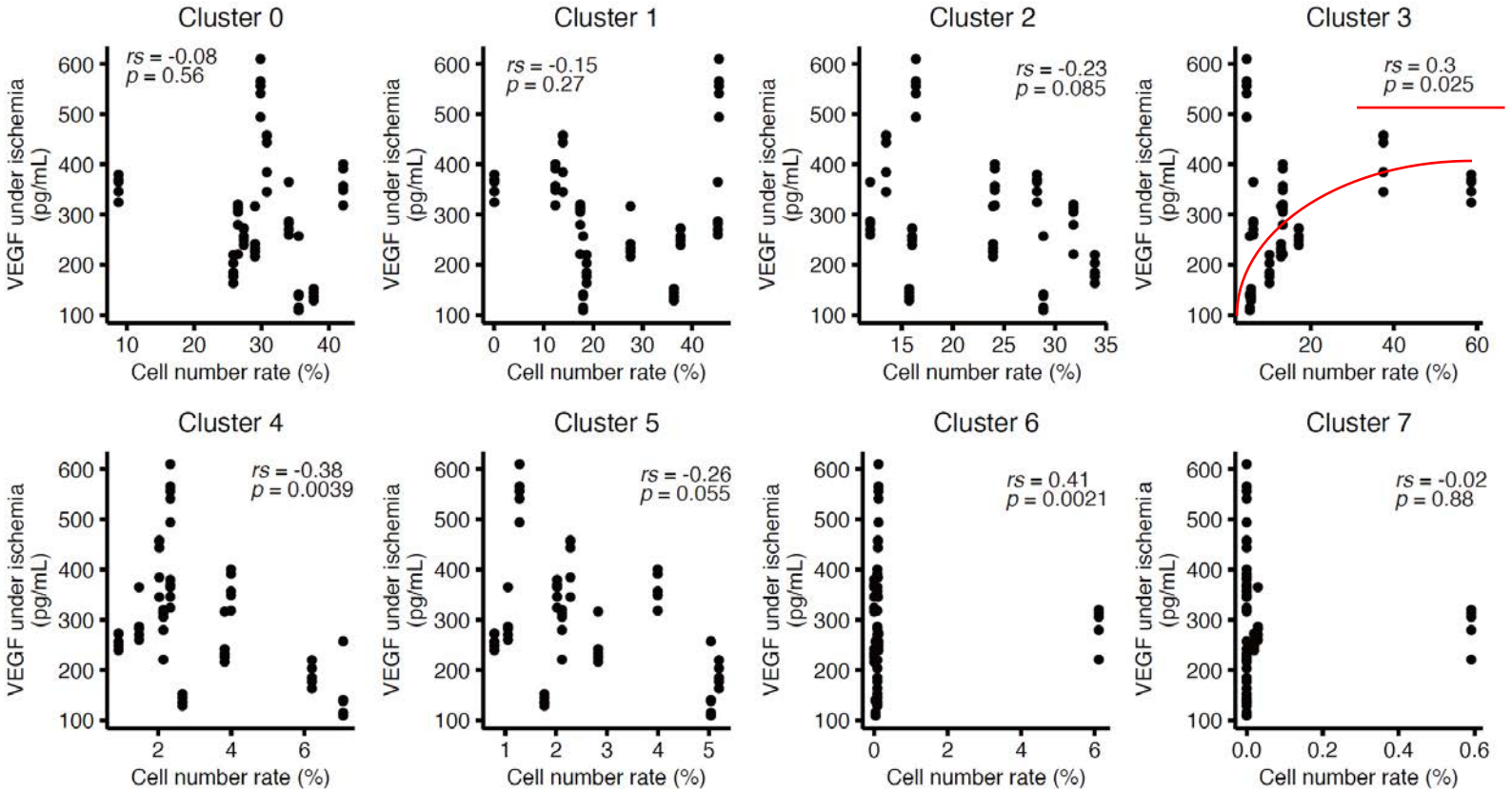
BM-MSCs (P5)
Normoxia



BM-MSC (P5)
Ischemia





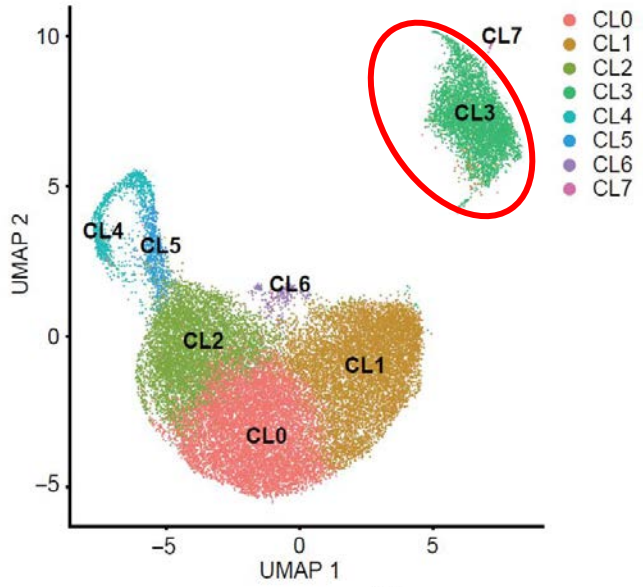


Miura T *et al., Stem Cells Transl Med.* 2023;**12**:379-390.

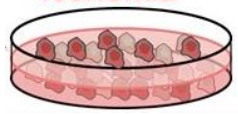
Functional involvement of LRRC75A

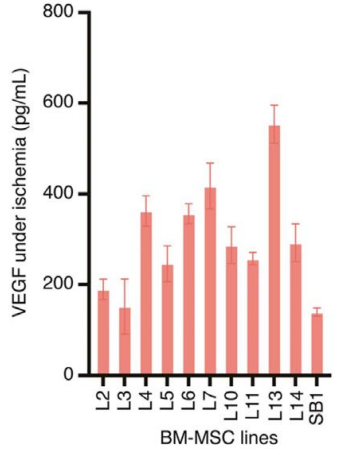
It is important to ensure this cell population, if you expect to reproduce angiogenesis and VEGF secretion!

BM-MSCs (P5)
Normoxia



BM-MSC (P5)
Ischemia

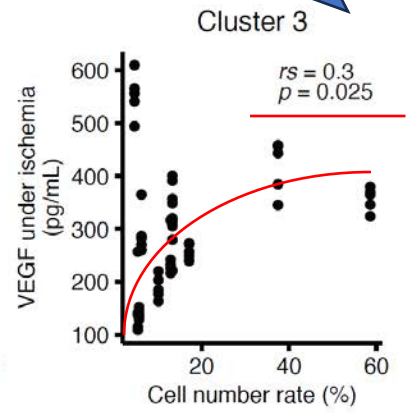




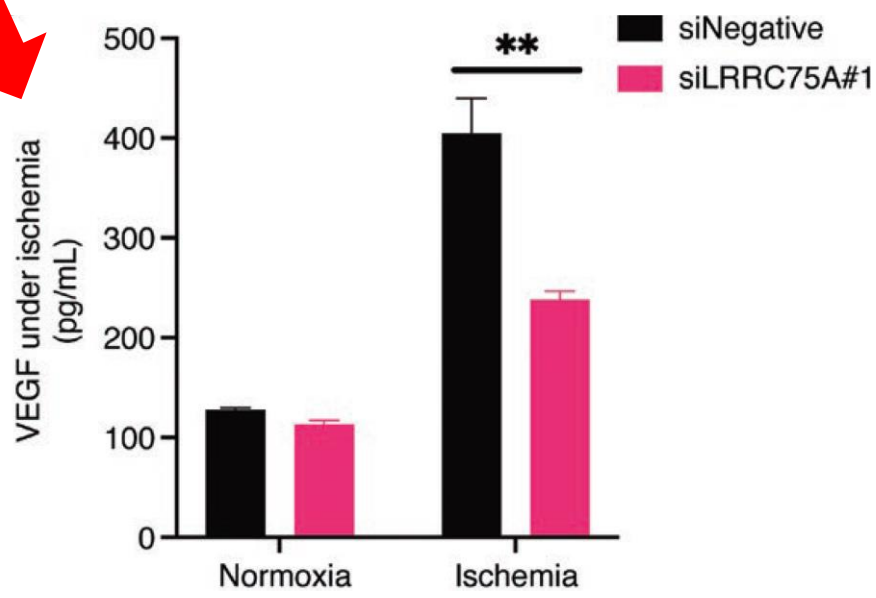
Top 20 upregulared genes of CL3

Gene name	Ave $\log_2$FC
LRRC75A	1.0357
KRT7	0.8382
KRT16	0.7902
C1orf56	0.7815
CRYAB	0.7696
HSPB1	0.7572
MTRNR2L12	0.7060
AC092069.1	0.7024
ADIRF	0.6712
LGALS1	0.6573
ID1	0.6525
MT2A	0.6424
S100A11	0.6312
COMP	0.6132
EIF5A	0.6057
FLG	0.6049
SH3BGRL3	0.5970
TPM2	0.5859
POLR2L	0.5555
GADD45B	0.5543

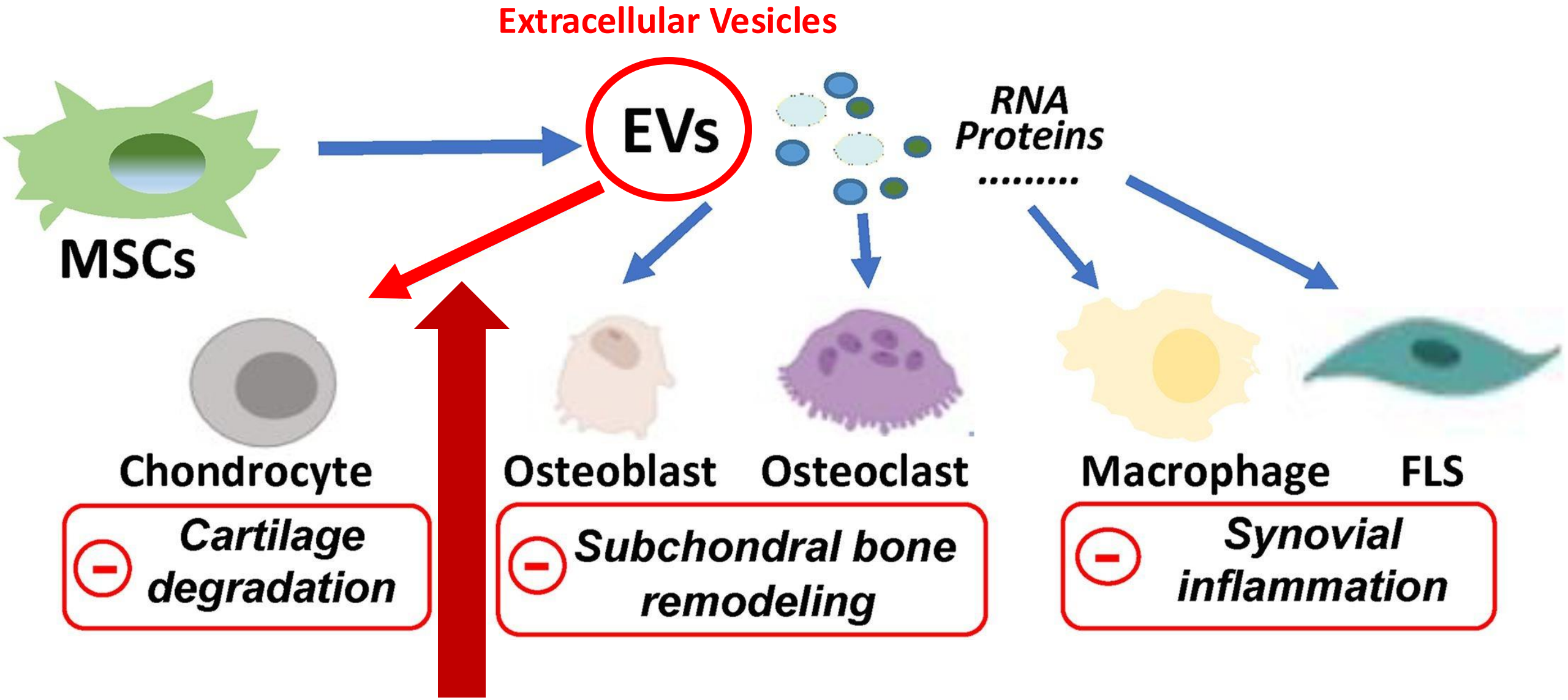
Hidden CQAs



***LRRC75A* is functionally involved in VEGF secretion during ischemia.**



EXAMPLE (B)



https://www.sciencedirect.com/science/article/pii/S0006295224002090

Chondrocyte Migration Assay (chemotaxis assay) to Evaluate the Chemotaxis-promoting Effect of hADSC-derived EVs

ClearView Chemotaxis Assay

1. Coat (migration) or prime (invasion) the insert

Prepare membrane surface for cell migration or invasion.

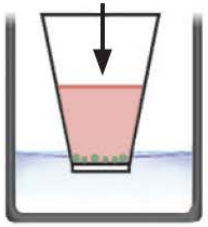
2. Harvest and seed (migration) or embed (invasion) cells

For migration, seed 1000-5000 cells and allow to settle.

For invasion, embed cells within matrix and centrifuge.

3. Treat cells

drug compound

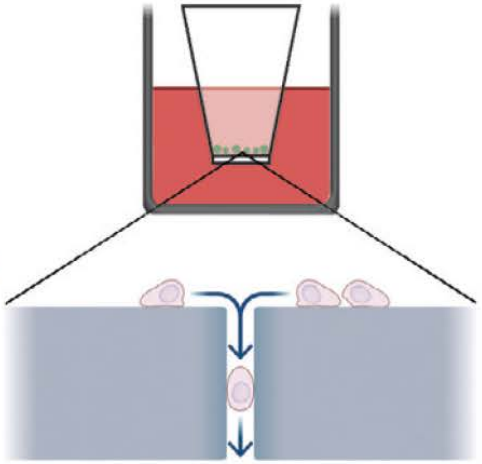


Add modulators of cell migration or invasion.

4. Add chemoattractant

Add chemoattractant or controls to reservoir plate wells.

5. Place in IncuCyte® and walk away



± EVs

Automatically collect time-lapse images.

Observe cell morphology.

Quantify migration and invasion.

Poster # P-07-01

https://www.sartorius.com/download/819566/cell-migration-and-invasion-assays-brochure-data.pdf

Effects of EVs derived from human adipose-derived MSCs (hADSCs) on the migratory activity of human chondrocytes

Unpublished data
論文未発表データ

EVs derived from human adipose-derived MSCs (hADSCs) promoted chondrocyte chemotaxis in a concentration-dependent manner.

Poster # P-07-01

Effects of EVs derived from human adipose-derived MSCs (hADSCs) on the migratory activity of human chondrocytes

Unpublished data
論文未発表データ

The area-under-curve (AUC) was calculated to quantify the migration-promoting activity (potency) as a single parameter from the effects of the EVs at the multiple concentrations.

Poster # P-07-01

Single-Cell Transcriptome Experiments

scRNA-seq results for 7 lots of hADSCs

Unpublished data
論文未発表データ

Poster # P-07-01

The population of hADSCs was estimated to consist of 9 clusters

Functional association between the effect of EVs and the hADSC clusters

It may be important to ensure cells of Cluster F, if you expect to reproduce the effect of hADSC-derived EVs on chondrocytes!

Unpublished data
論文未発表データ

The population size of Cluster F correlated significantly with the migration-promoting effect of the EVs on human chondrocytes.

Poster # P-07-01

Summary

- Because of the complexity and inhomogeneity of the raw materials and active ingredient cells in CTPs, even if all recognizable quality attributes were listed, it would be impossible to identify and adequately control all of the quality attributes (CQAs) that are important to ensure reproducibility of product efficacy and safety.
- Identifying the "true active cells" of a CTP, identifying hidden CQAs, and understanding the mechanism of action requires a science to classify and understand the inhomogeneity within a cell population (e.g., subpopulations of MSCs) based on their potency and efficacy, which may be called "Stem Cell Pharmacotaxonomy."
- This may lead to the acquisition of intellectual property (new mechanism of action and active ingredients) and good drug prices (additional breakthrough and usefulness).
- In other words, the value of CTPs can be expected to increase, and quality and supply stability/continuity can be expected.
- 細胞加工製品の原料や有効成分である細胞は複雑で不均一であるため、認識しうる品質特性をすべて列挙したとしても、製品の有効性や安全性の再現性を保証するために必要十分な重要品質特性(CQA)をすべて特定・管理することはできないかもしれない。
- 細胞加工製品の「真の有効細胞」の特定、隠れたCQAの同定、および作用機序の理解には、細胞集団内の不均一性(例:MSCの亜集団)をその効力・有効性にもとづき分類・理解するための科学、言わば、Stem Cell Pharmacotaxonomy「幹細胞薬理分類学」が必要。
- これらは、知財(新規作用機序・有効成分)や薬価(画期性加算・有用性加算)の獲得にも繋がりうる。
- つまり、細胞加工製品の価値向上、品質や供給の安定性・継続性が期待できるということです。

ACKNOWLEDGMENTS

Collaborators

Takumi Miura[1,2,3], Tsukasa, Kouno[3,4], Megumi Takano[1], Takuya Kuroda[1,3], Yumiko Yamamoto[4], Shinji Kusakawa[1], Masaki Suimye Morioka[4], Tohru Sugawara[2,5], Takamasa Hirai[1], Satoshi Yasuda[1,3], Rumi Sawada[1], Satoko Matsuyama[1,6], Hideya Kawaji[4,7], Takeya Kasukawa[4], Masayoshi Itoh[4], Akifumi Matsuyama[6], Jay W. Shin[4,8], Akihiro Umezawa[2], Jun Kawai[3,4] , Takamasa Hirai[1], Hirotaka Nishimura[9], Tomofumi Yamamoto[9], Akiko Ishii[9]

[1] Division of Cell-Based Therapeutic Products, **National Institute of Health Sciences (NIHS)**, Kanagawa, Japan

[2] Center for Regenerative Medicine, **National Center for Child Health and Development (NCCHD)**, Tokyo, Japan

[3] Life Science Technology Project, **Kanagawa Institute of Industrial Science and Technology (KISTEC)**, Kawasaki, Japan

[4] **RIKEN Center for Integrative Medical Sciences**, Yokohama, Japan

[5] Biopharmaceutical and Regenerative Sciences, Graduate School of Medical Life Science, **Yokohama City University**, Yokohama, Japan

[6] Center for Reverse TR**, Osaka Habikino Medical Center**, Osaka Prefectural Hospital Organization, Osaka, Japan

[7] Research Center for Genome & Medical Sciences, **Tokyo Metropolitan Institute of Medical Science**, Tokyo, Japan

[8] Genomic Institute of Singapore, **Agency for Science, Technology and Research (A*STAR)**, Singapore

[9] Division of Biological Chemistry and Biologicals, **National Institute of Health Sciences (NIHS)**, Kanagawa, Japan

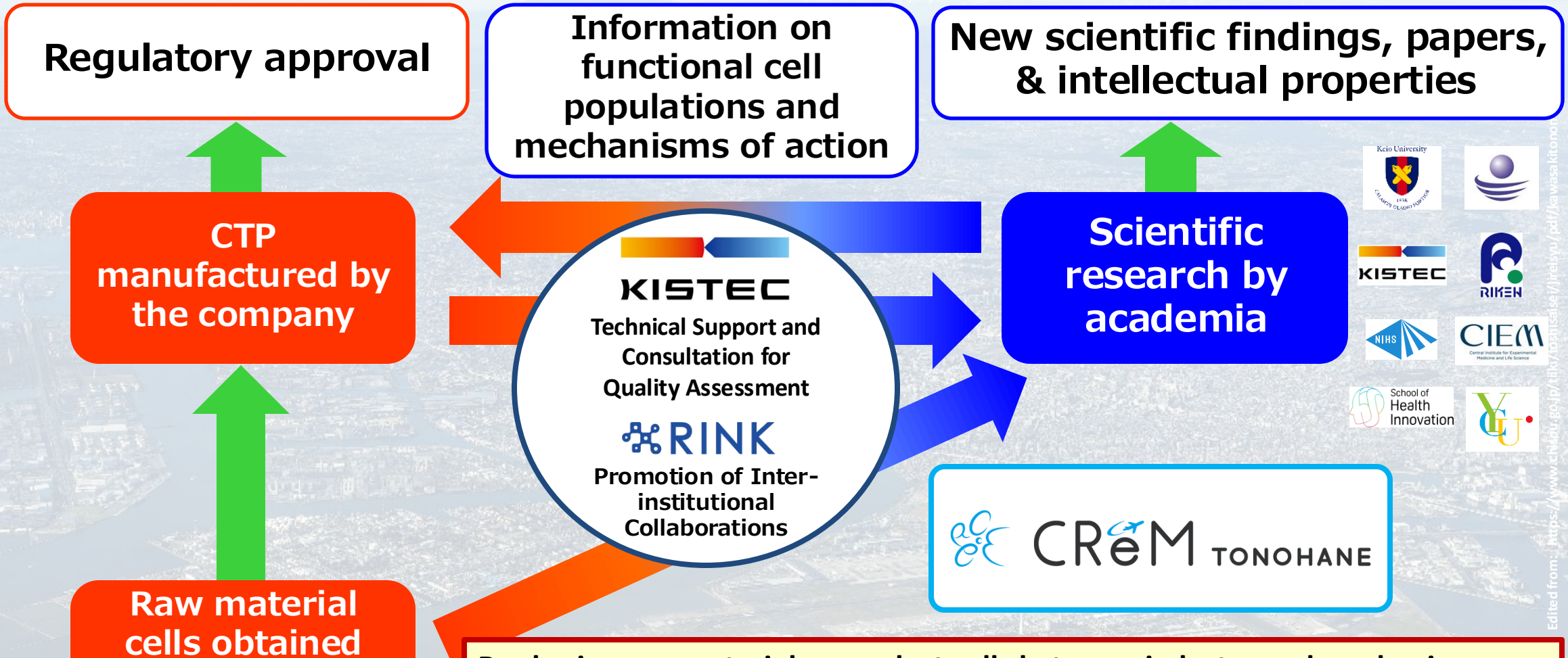
Funded by

AMED

神奈川県 Kanagawa Prefectural Government

Edited from: https://www.chisou.go.jp/tiiki/toshisaisei/jireisyu/pdf/kawasakitonomachi_jirei.pdf

“Visualization” of heterogeneity/inhomogeneity and understanding of quality, mechanism of action, and product value of raw material cells and active ingredient cells



By sharing raw material or product cells between industry and academia, companies may be able to use information on functional cell populations and mechanisms of action obtained in academia to ensure the quality of their CTPs.
→ possibly leading to their product value and flexible cell manufacturing

For more information about the technical assistance/consultation for quality assessment of cell therapy products by CReM TonoHane's Quality Assessment Team, please contact:

Life Science Technology Project,

Kanagawa Institute of Industrial Science and Technology

地方独立行政法人 神奈川県立産業技術総合研究所（KISTEC）

次世代ライフサイエンス技術開発プロジェクト

sm-pg-shiken@kistec.jp

KISTEC

Edited from: https://www.chisou.go.jp/tiiki/toshisaisei/jireisyu/pdf/kawasakitonomachi_jirei.pdf