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Scientific Challenges for the Safety, Efficacy and Quality of Cell-based Therapeutic Products

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Points to Consider on Manufacturing Process & Quality of CTPs

- Characterization and understanding of specific profiles of cells at critical steps(starting, intermediate, final) and their eligibility (differences in autologous/ allogeneic)
- Eligibility of other raw materials and manufacture-related substances and their quality control (especially, eligibility of biological materials, no adverse impact of non-cellular/tissue component on desired cells)
- Verification of manufacturing process and constancy of manufacture
- Product consistency in terms of quality attributes such as identity, purity, homogeneity and potency
- Stability (storage conditions/expiration date, freezing & thawing processes, and shipping vessel & procedure)
- Quality control of final product through relevant combination of critical quality elements from products & process aspects

Points to Consider on Non-clinical Efficacy

- Examination of functional expressions, persistence of C/T action and their expected therapeutic efficacy through appropriately-designed tests using relevant animals and cells (POC)
- Examination of therapeutic effects using cell/tissue models or disease-model animals, where appropriate and possible
- Indication of far more promising effect or performance of the product than other medical treatments
- Evaluation of Benefit vs. Risk, considering about Potential Risk of Product vs. Real Risk of Patient

Points to Consider on Non-clinical Safety

- Presence of microorganism, especially Viruses
- Tumorigenicity (ESC/iPSC-derived products)
- Inappropriate differentiation, ectopic tissue formation, undesired phenotype (SSC/ESC/iPSC-derived products)
- Immunogenicity, immune rejection or other unanticipated immune responses (allogeneic CTPs)
- Testing in relevant animal models or *in vitro* to a technically possible and scientifically reasonable extent, by taking into account the nature of the product and its target disease.
- Testing cells/tissue models of animal origin in relevant animal models, if such product models that can mimic those of human are available.
- No adverse impact of non-cellular/tissue components in starting materials or products
- Evaluation of risk vs. benefit, taking into consideration about potential risk concerns of product vs. real risk of patient

“Tumorigenicity”

The capacity of a cell population inoculated into an animal model to produce a tumor by proliferation at the site of inoculation and/or at a distant site by metastasis.

Reference

WHO Technical Report Series 978 Annex 3 “Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks” (2013)

International Guidelines for Tumorigenicity Testing

- **WHO Technical Report Series 978 Annex 3 "Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks" (2013)**

… is for QC of cell substrates like CHO cells and HEK293 cells

… and excludes viable animal cells when they are used directly for therapy by transplantation into patients or when they are developed into cell lines for the purpose of using them as therapeutic agents by transplantation

- **There is no international guideline document for tumorigenicity testing of CTPs.**

New product must be evaluated, based on new concepts

“New wine must be put into new bottles”

Purposes of Tumorigenicity(-Associated) Testing for CTPs

1) Quality control of cell substrates (i.g., ESCs, iPSCs)

Tumorigenicity is a critical quality attribute of **homogeneous cell substrates**.

・・・WHO TRS 978 is applicable

2) Quality control of intermediate/finished products during manufacturing processes

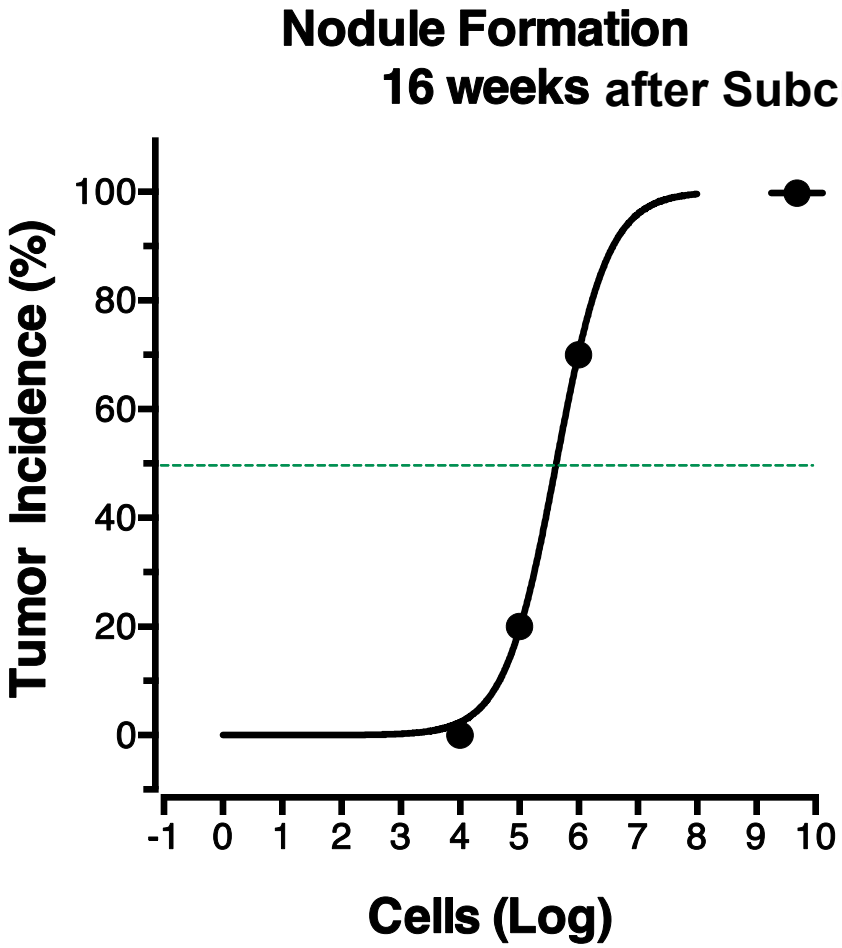
The amount of tumorigenic cellular impurities is one of critical quality attributes.

・・・LOD is the Key

3) Non-clinical safety assessment of finished products

Tumorigenicity in the site of administration site is estimated, by in vivo tumorigenicity testing with immunodeficient animals

Sensitivity of Tumorigenicity Testing with Nude Mice (The Method in WHO TRS 978)



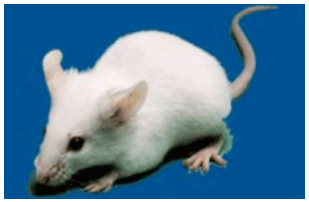
Dose to form a tumor in 50% of the animals

	TPD50
Nude	4.0×10^5

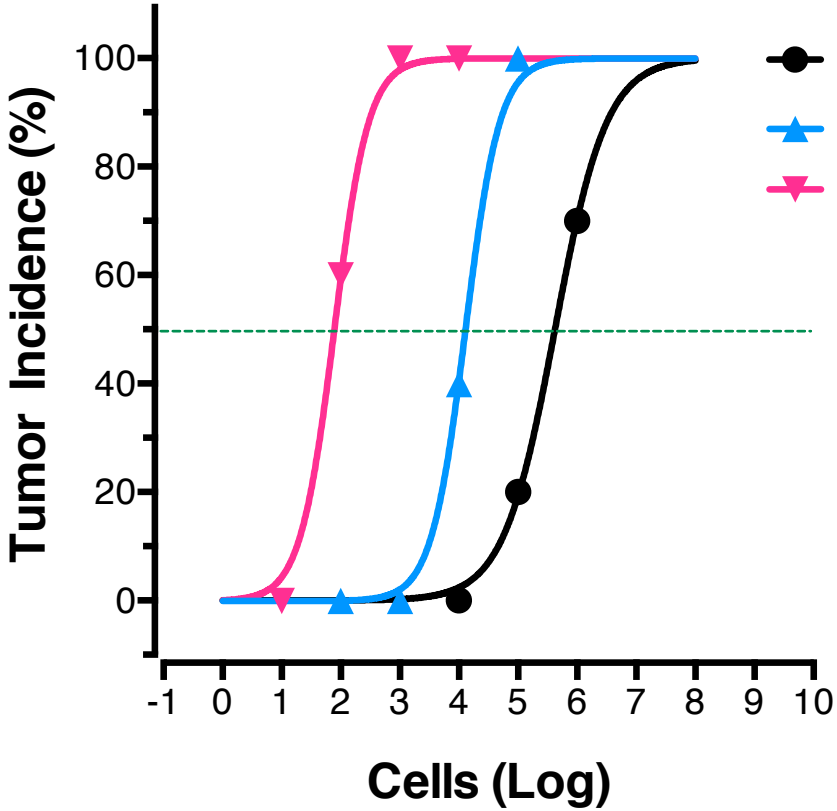
The sensitivity is not sufficient for CTPs

Kusakawa *et al., Regen Therapy* 2015;1:30-7.

In Vivo Tumorigenicity Tests for HeLa Cells with NOG Mice and Matrigel



Nodule Formation
16 weeks after Subcutaneous Administration



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

Kusakawa *et al., Regen Therapy* 2015;1:30-7.

Detection of Tumorignic Cellular Impurities (HeLa) in Normal Cells (hMSCs) by NOG mice and Matrigel

Kusakawa *et al., Regen Therapy* 2015;1:30-7.

Strain	Group	Tumor incidence at indicated HeLa cell dose at week 16					TPD_{50} at week16
		0	1×10	1×10^2	1×10^3	1×10^4	
NOG	HeLa/hMSC (1×10^6)	0/6	0/6	3/6	6/6	6/6	1.0×10^2
NOG	HeLa/hMSC (1×10^7)	0/6	1/6	2/6	-	(6/6)[a]	1.8×10^2

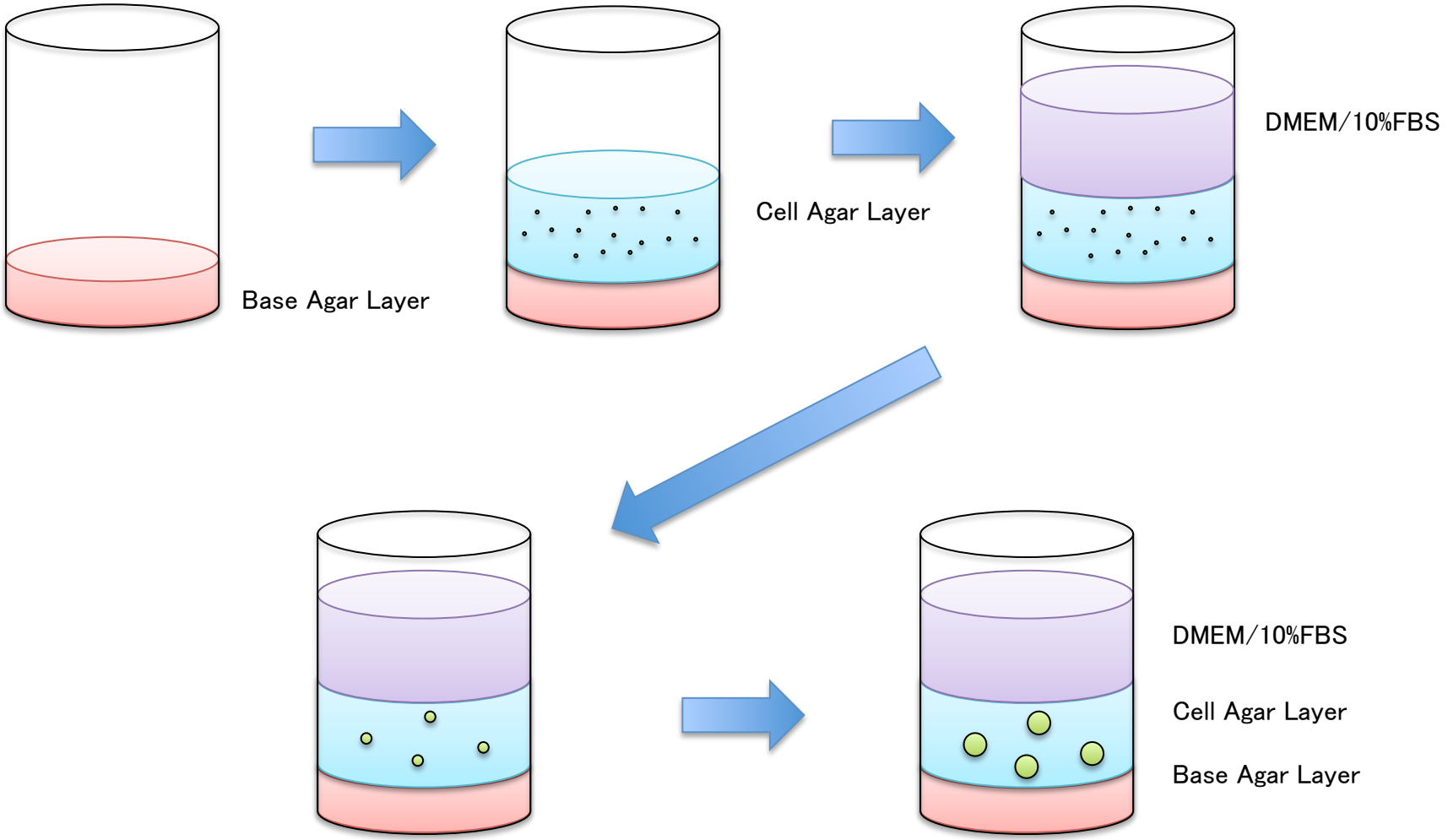
a: Since not all animals inoculated with the highest dose (10^2) have formed tumors, it was assumed that the tumor incidence of animals at an even higher dose step (a dummy set of data) would have been 100%.

-: Not tested; ND: Not determined

This method detects HeLa cells in hMSCs
at ratios of approx. $1/10^4$ and $1/10^6$, at probabilities of 50% and 17%, respectively.

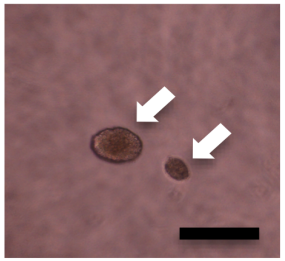
If the acceptable false negative rate is 1%, sponsors need to confirm no tumor formation in [log0.01/log(1-0.17)=] 25 mice inoculated with 10^7 hMSCs, to show that the ratio of HeLa-like cellular impurities to hMSCs are less than $1/10^6$.

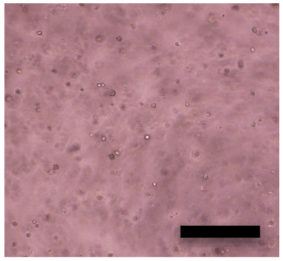
Soft Agar Colony Formation Assay



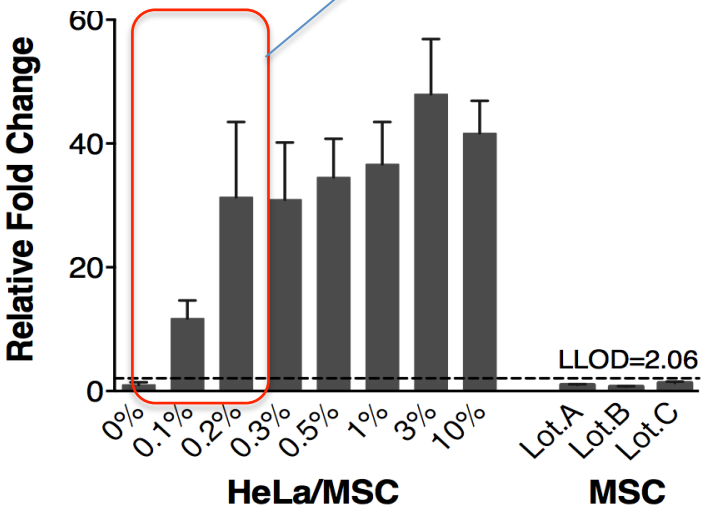
Detection of Tumorigenic Cellular Impurities (HeLa) in Normal Cells (hMSCs) by Soft Agar Colony Formation Assay

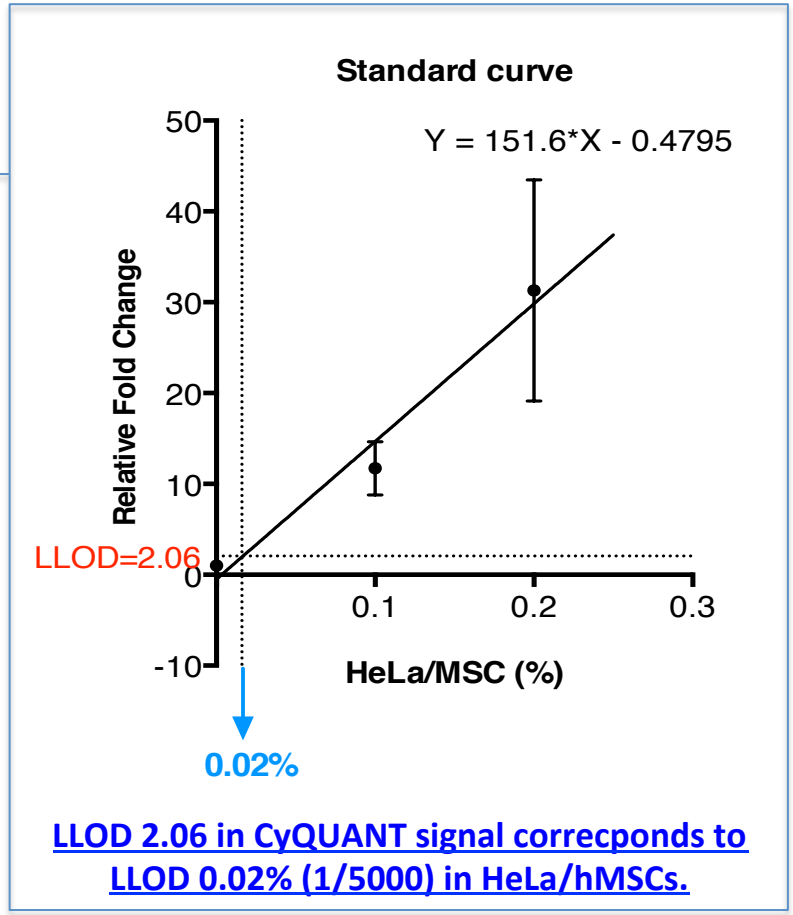
Soft-Agar Colony Formation Assay (20 days) → detected 0.1% (1/1000) HeLa/hMSCs※





* Quantitation by DNA-Binding Dye (CyQUANT)





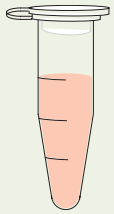
LLOD 2.06 in CyQUANT signal correcponds to LLOD 0.02% (1/5000) in HeLa/hMSCs.

Kusakawa *et al., Regen Therapy* 2015;1:30-7.

“Digital” Soft Agar Colony Formation Assay

Highly Sensitive Method for Quantitation of Tumorigenic Cellular Impurities in CTPs By Digital Counting of Single Tumorigenic Cells

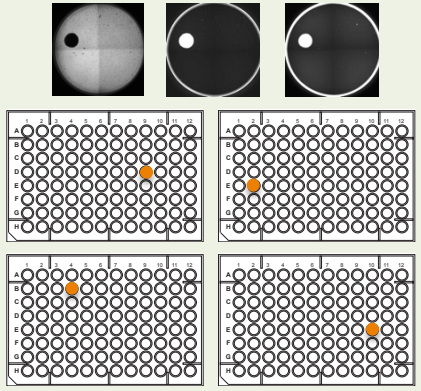
Cell Preparation



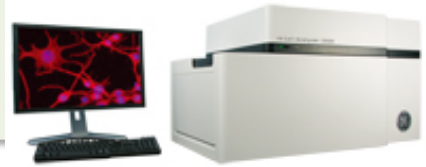
Sample Partitioning

※ A Sample Image

Colony Counting by High-Content Imaging

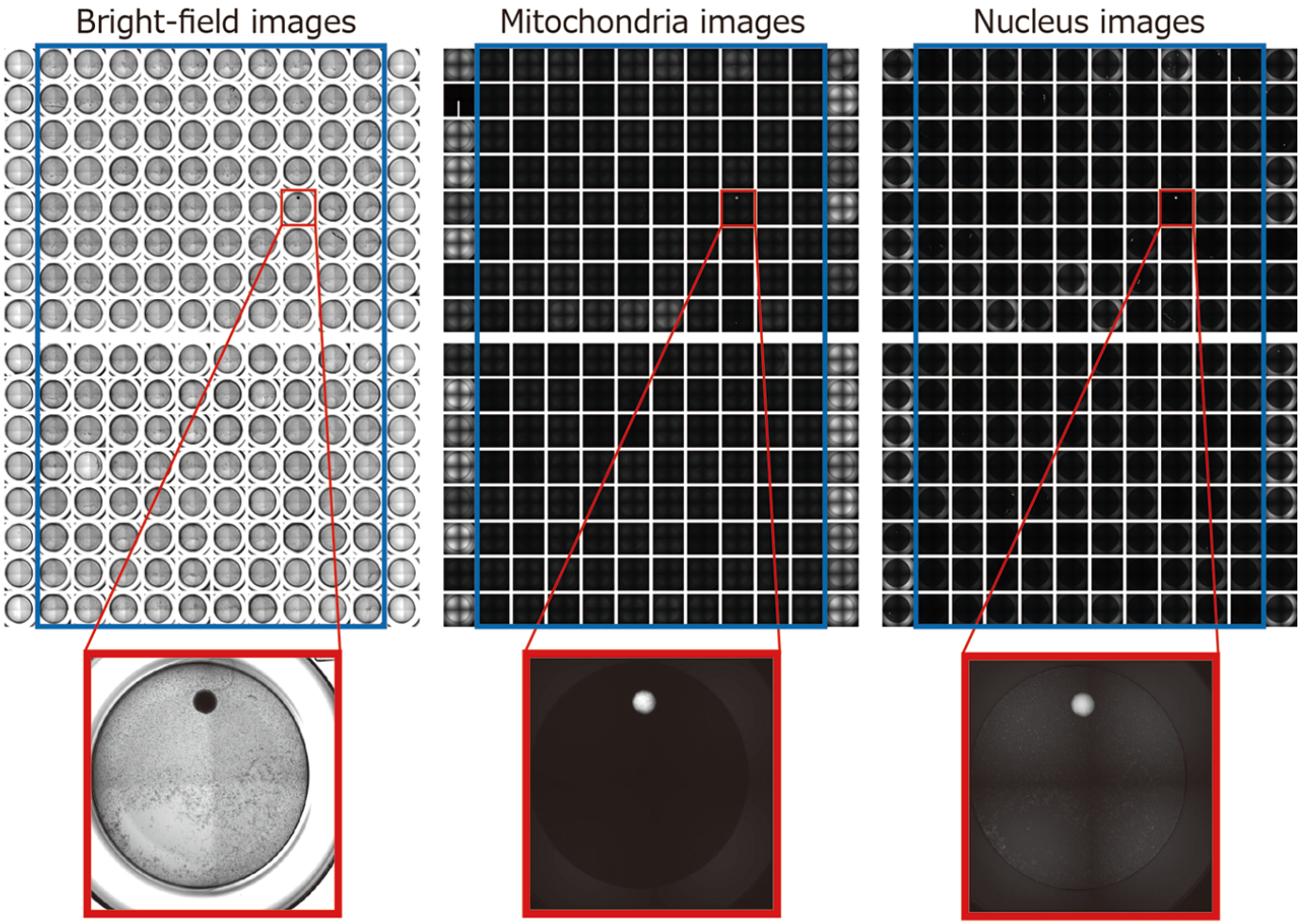


positive well



High-throughput imaging with the *IN Cell Analyzer 2000*

Cell preparation : HeLa 1 / MSC 10,000,000 → 160wells (HeLa 0.0125 / MSC 62,500 / well)



When a cell suspension containing a single HeLa cell and 10^7 hMSCs was aliquated into 160 wells and cultured in the soft agar media, one "positive" well was detected, indicating its ability to detect as low as 0.00001%（1/10,000,000）HeLa cells in hMSCs.

Kusakawa *et al., Sci Rep.* 2015; 5: 17892.

Conclusions

- The development of CTPs is uncertain, because they include advanced and emerging technologies with limited clinical experiences. One of the biggest problems is that evaluation tools and approaches to ensure their safety, efficacy and quality are often lacking.

- For example, tumorigenicity is one of the major concerns for developing CTPs, particularly human ES/iPS cell-based products. However, no detailed guideline has been issued for tumorigenicity testing for CTPs.

- We need to establish new concepts and testing methods for new Q/E/S issues of advanced products.

- By understanding the abilities and limitations of each testing method, we should select appropriate methods that meet the criteria for decision-making during development of CTPs.

HESI Annual Meeting

June 8, 2016

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