Safety of Cell Therapy Products: *In vitro* Methods to Assess the Tumorigenicity of Human Cell-Based Therapeutic Products

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The challenge - Tumorigenicity assessment of human Cell-Based Therapeutic Products

Human pluripotent stem cells (hPSC) have the potential to revolutionize regenerative medicine, however:

- There are **concerns** associated with hES/iPSC-derived products (MSC/HSC, CAR-T): 1) the possibility of **residual undifferentiated PSCs** in the final product and 2) cell transformation occurring during the manufacturing process, which could lead to **tumorigenicity**;
- Currently, there is **no globally accepted consensus on** the evaluation of methods for **tumorigenicity *in vivo* or *in vitro*,** leading to high variability of data presented in regulatory submissions and difficulty in interpretations;
- A public-private partnership (PPP) initiative to evaluate/standardize existing methods for detection of undifferentiated cells and transformed cells is critically important not only for product developers but also for regulatory authorities and patients;
- The Health and Environmental Sciences Institute (HESI)' **CT-TRACS** Committee has launched a call for participants to form an **International Multi-Site Study** to test *in vitro* methodologies for tumorigenicity assessment of cell therapy products.

HESI CT-TRACS: Cell Therapy - TRAcking, Circulation, & Safety (CT-TRACS) Committee

Mission: To improve the safety of cell-based therapies for patients by enhancing our ability to reliably apply analytical methods, devices, and scientific knowledge to evaluate the distribution and fate of these cells in a patient.

International, Multi-Stakeholder & Multi-Disciplinary Collaborative Effort

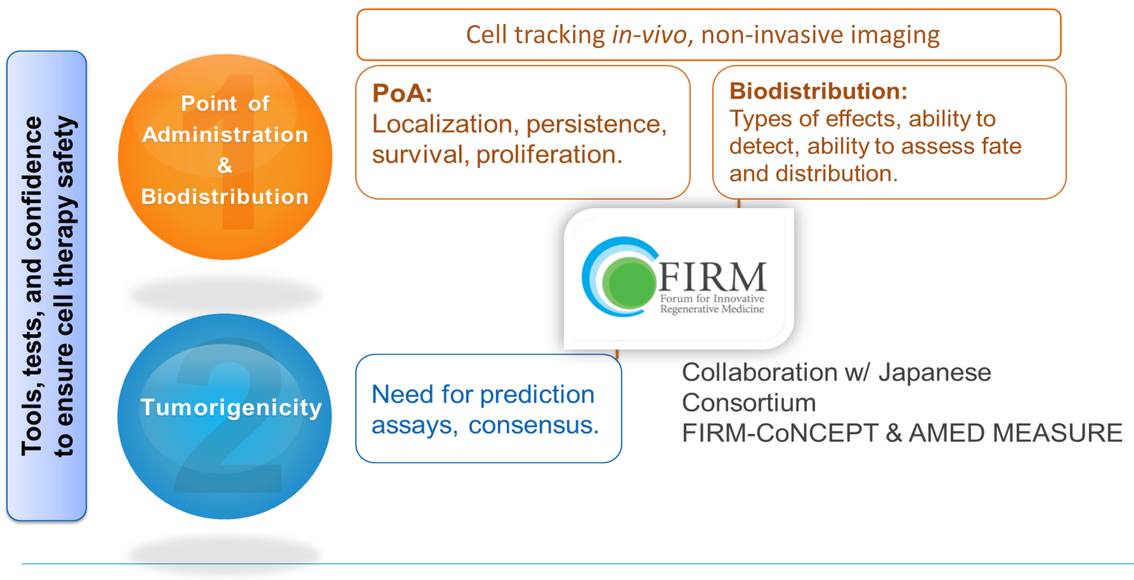
Regulatory bodies: FDA, cBG MEB Medicines Evaluation Board, MHRA

Universities/ Research Centers: King's College London, Memorial Sloan Kettering Cancer Center, Radboud University, UCL

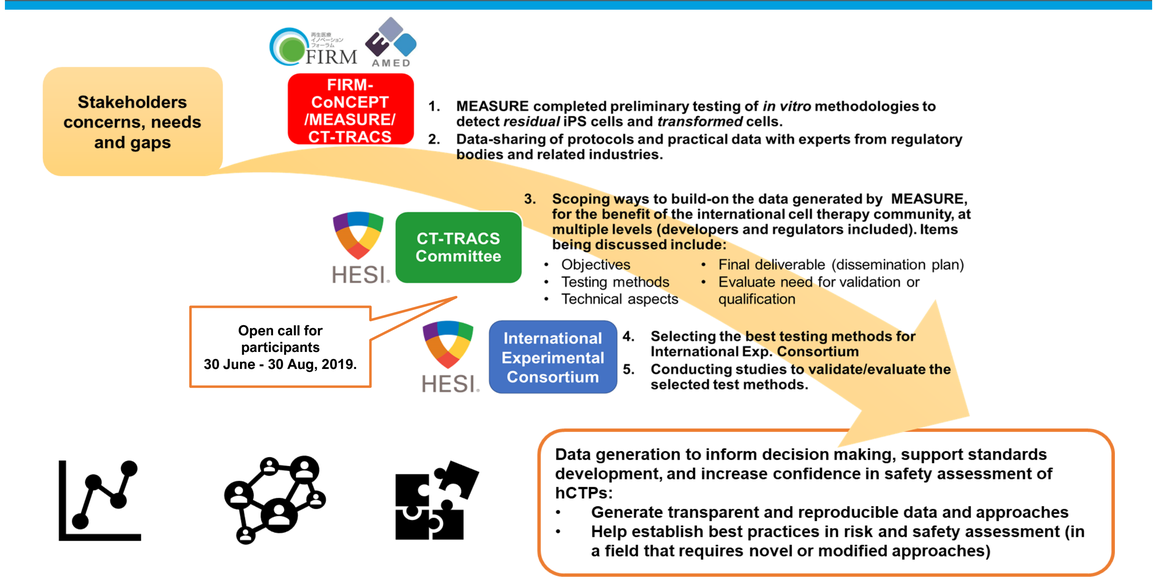
Gov./Consortia/NGO: CATAPULT Cell Therapy, eatris, FIRM, NIH National Institutes of Health, NIHS, RIVM, National Institute for Public Health and the Environment, PACT Production Assistance for Cellular Therapies National Heart Lung and Blood Program

Industry: Astellas, Athersys, Boehringer Ingelheim, Cellular Dynamics International, Celsense, charles river, GE Healthcare, janssen, NOVARTIS, Sumitomo Dainippon Pharma, TACONIC, Takeda, VisiCELL MEDICAL

CT-TRACS work groups



Strategy to address challenges & needs[1]



[1] Sato *et al.* 2019. Tumorigenicity assessment of cell therapy products: The need for global consensus and points to consider *(accepted to Cytotherapy)*

International Multi-Site replication study to assess *in vitro* methods for tumorigenicity

CT-TRACS' Tumorigenicity WG has identified "*in vitro*" methodologies as a priority need, due to: **1.** concerns about the practical use of animals in testing doses relatable to human application; **2.** concerns about the relevance of testing human cells in animals; **3.** interest in developing alternative methods contributing to the 3Rs of animal use in R&D.

Goals:

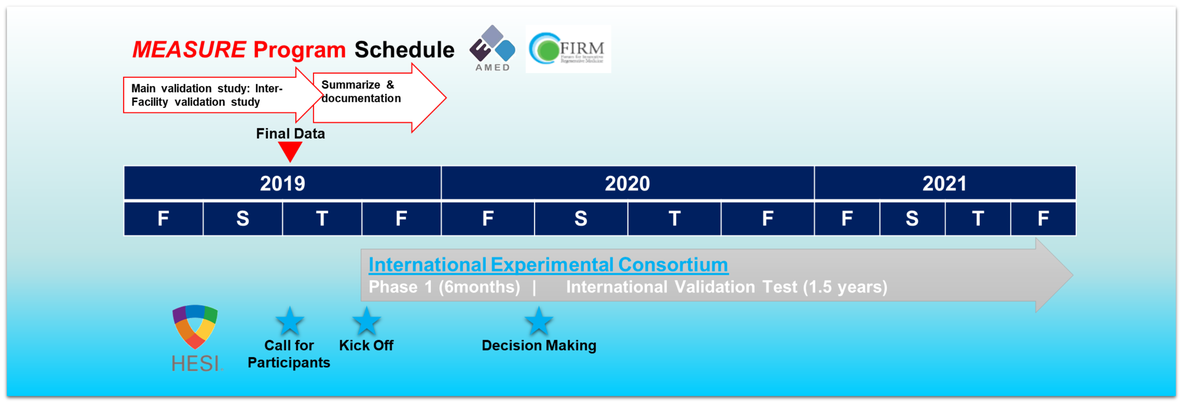
- ✓ Develop better, standardized *in vitro* methods for predicting tumorigenicity;
- ✓ Engage regulators to ensure that the models and data generated through the initiative will address regulatory safety concerns;
- ✓ Aid researchers, developers and regulators assess the safety of CTPs with more confidence and contribute to faster/earlier decision-making;

Assays:

Multiple types of *in vitro* methods to assess residual pluripotent stem cells in PSC-derived products are being considered with high priority.

Assays/ Platform	Flow cytometry 	qRT-PCR 	Droplet Digital PCR 	Direct detection using a highly efficient amplification method*
Positive control	iPS cells	iPS cells	iPS cells	iPS cells
Duration	1 day	6 hours	a few hours	about a week
Marker	TRA-1-60 etc	Lin28	Lin28	-
Pros	Simple/quick	Simple/quick, High sensitivity	Simple/quick, High sensitivity	Direct detection, High sensitivity
Cons	Low sensitivity, Indirect detection, Difficulty in the manual selection of marker thresholds	Indirect detection, Lin28 expression is noted in some differentiated cells	Indirect detection, Lin28 expression is noted in some differentiated cells	Time-consuming, Low throughput
Sensitivity	0.1%	0.002%	0.001%	0.01-0.001%
Reference	Kuroda et al., PLoS ONE. 2012	Kuroda et al., PLoS ONE. 2012	Kuroda et al., Regen Ther. 2015	Tano et al., PLoS ONE. 2014

Proposed timeline:



How can I get involved?

1) **Join! The CT-TRACS program** is open to new participants with relevant technical expertise, from both public and private sectors. The program also seeks creative funding partners and encourages inquiries by those with interest in providing financial support for these innovative efforts. For committee information, visit: www.hesiglobal.org/cell-therapy-tracking-circulation-safety-ct-tracs or contact Lucilia Mouriès, Sc. Program Manager, at: lmouries@hesiglobal.org.
2) **Contribute to the design of a new database in development by completing our survey.** The database aims to be a resource to identify ways of tracking cell therapies *in vivo,* non-invasively, to inform about safety and efficacy. *Link*: https://bit.ly/2Xt1uFQ , or *QR code*:

We work with Innovate UK

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