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

CALL FOR PARTICIPANTS:
International Multi-Site Study - *In vitro* Methods to Assess the Tumorigenicity of Human Cell-Based Therapeutic Products

The Health and Environmental Sciences Institute' CT-TRACS Committee is opening a call for participants to form an International Multi-Site Study to test *in vitro* methodologies for tumorigenicity assessment of cell therapy products (CTPs).

Open date: June 30, 2019 – Application deadline: August 30th, 2019

Project start date: 27 September 2019, Kickoff meeting in person (HESI facilities, Washington DC).

Title: "Predictive Methods to Assess the Tumorigenicity of Human Cell-Based Therapeutic Products *In vitro*: A multi-site replication study"
Initiators: HESI CT-TRACS Committee; Contact: Lucilia Mouriès, Scientific Program Manager; lmouries@hesiglobal.org
Preliminary materials from MEASURE study: http://hesiglobal.org/event/cell-and-gene-therapy-catapult-seminar-series-ct-tracs-workshop/ (in particular, presentations from: Dr. Keiji Yamamoto, Takeda/FIRM, Japan; Dr. Yoji Sato, NIHS, Japan; Dr. Takatoshi Koujitani, Sumitomo Dainippon Pharma, Japan).
To express your interest: please complete the form in Appendix-1 and submit to Lucilia Mouriès (lmouries@hesiglobal.org) by August 30th, 2019, accompanied by your biography and a brief statement describing: the reason of your interest; how you/your organization expertise relates to the project (e.g., current or past experience developing similar methods; tumorigenicity assessment experience); and the type of support you anticipate your organization will be able to provide to the study (see paragraph "HOW?").

WHO?
Organizations from the public and private sector interested in participating in the experimental multi-site international consortium.

WHY?
- Human pluripotent stem cells (hPSC) have the potential to revolutionize regenerative medicine.
- However, there are concerns associated with hES/iPSC-derived products (and to some extent, MSC/HSC, CAR-T etc.), in particular, the possibility of residual undifferentiated PSCs persisting in the final product, and/or cell transformation occurring during the manufacturing process, both of which could lead to tumorigenicity (Herberts et al., 2011; Bailey, 2012; Basu et al., 2015).
- Currently, there is no globally accepted consensus on the evaluation methods for tumorigenicity *in vivo* or *in vitro*.

- Product developers have to date established customized assays in-house and tested their products on a case-by-case basis. This results in a high variability of data presented in regulatory submissions and difficulty in interpretations.
- A public-private partnership (PPP) initiative to evaluate the 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.

Rationale:

***MEASURE:** ***M**ultisite **E**valuation Study on **A**nalytical Methods for Non-clinical **S**afety Assessment of h**U**man-derived **RE**generative Medical Products.,* is a Japanese PPP initiated in 2016, aiming at validating various methodologies (both *in vivo* and *in vitro*) for the ability to detect residual undifferentiated cells or transformed cells, through collaborative efforts between industry, CROs, academia and the Japanese regulatory authorities.

To our knowledge, there is no similar initiative at the international level at this time. A multi-site international initiative would increase the technical capability of researchers, increase the quality of assays, and allow developers/regulators around the world to understand the tumorigenic potential of the cell therapy products based on the results from established assays which could be applied consistently.

WHAT?

Evaluation of *in vitro* testing methods to address tumorigenic potential of the pluripotent stem cell-derived products, through an international consortium, with the support of the HESI CT-TRACS committee. Focus on iPSC.

This proposal aims to build-on knowledge generated from the MEASURE studies and present a path towards their international evaluation, with a goal to engage key stakeholders from regulatory agencies, standards organizations and other scientific organizations involved in developing pluripotent stem cell-based therapies.

CT-TRACS has identified "*in vitro*" methodologies as a priority need, for three main reasons:

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 (translatability);
3) Interest in developing alternative methods contributing to the 3Rs of animal use in R&D.

Therefore, this proposal focuses on *in vitro* methodologies only.

Project objectives:

- Develop better, standardized *in vitro* models for predicting tumorigenicity.
- Engage regulators to ensure that the models and data generated through the initiative will address regulatory safety concerns and will provide the necessary information to support regulatory filings.
- Generate new data to fill the knowledge gaps for the field through a coordinated effort.
- Contribute to the development of (currently lacking) standardized approaches for the assessment of CTPs.

- 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 (see Appendix 1 for a general description of the methods).
- Cell transformation assessment assays may be considered in the future if stakeholders express significant interest in undertaking these.

Deliverables: "Know-How" of the experimental methods and best practices; publication of scientific articles.

Project duration: based on the MEASURE preliminary studies conducted in Japan, it is expected that these experiments will be conducted over a 1.5- to 2-year time frame.

Project phases:

1. **Phase 1: "Discussion Phase" (~ 6 Mo)** - The initial phase will consist of team discussions with all participants to: select methods to be evaluated, agree on protocols, assay design and conditions, acceptance criteria, endpoints, measures of success, and set a timeline.
   - Test cells and control cells: one of the first elements of discussion will be to decide the type of cells to be used (e.g., differentiated cell from iPSC) spiked with undifferentiated cells (e.g., iPSCs). The best approach will be defined during the "Discussion Phase", taking into consideration feedback received from stakeholders during the initial "Data Sharing Phase" of the MEASURE preliminary results.
2. **Phase 2: Protocol optimization (~ 6 Mo)** - Researchers conducting the experiments will gather and discuss protocol refinements. The protocols and data obtained from "MEASURE" will be shared.
3. **Phase 3: Study Phase (~ 6 Mo)** – assays are conducted at each site; all sites will follow conditions agreed upon during Phase 1.
4. **Phase 4: "Reporting Phase" (~ 3 Mo):** report results and discussion/interpretation with all participants in the consortium.
5. **Dissemination:** manuscripts(s) development; report at conferences/workshops.

Eligible candidates: cell therapy developers, CROs, academic research institutions, government laboratories and other organizations with capabilities to conduct hands-on testing of the proposed methodologies.
At least 3 organizations (preferably more) for each selected assay, are needed to conduct the study based on a pre-defined protocol, agreed upon by all participating organizations during the scoping phase.

HOW?

Participants can contribute in several ways:

- Technically conduct the study in house: provide reagents, materials & equipment and human resources to conduct the experiments (in-kind) - *note: special equipment may be required - specific details on methodologies and equipment are listed in table 1.*

- Contract (and financially support) a CRO to conduct the study, per the protocols and conditions agreed upon as a group.
- Provide cells to be used in the experiments (e.g., control cells; test cells)
- Provide expert input throughout the duration of the studies (e.g., regulators; scientific advisors).
- Provide funding to enable the realization of the study.
- Provide expertise for and/or undertake the data analysis, statistical analysis etc., by the criteria adopted by the group.

The Project will be conducted as part of the HESI CT-TRACS Committee' Tumorigenicity WG, with infrastructure support from HESI for program coordination.

Requirements - To be considered, participants will need to commit to:

1. Financially support the test(s) conducted at their site – materials, reagents and man-hours for labor - (or identify funding mechanisms to support those).
2. Identify one or more scientist(s) to lead the project at their site and be the liaison with CT-TRACS until conclusion of the project (involves time commitment for regular participation in monthly teleconferences to discuss project progress and potential F2F meetings).
3. Commit to join (or continue participation in) the CT-TRACS Committee for the duration of the Project. HESI will provide the infrastructure for the efforts to take place (e.g. regular teleconferences among participants, coordination, data sharing, and dissemination of the results). Participation in the CT-TRACS committee involves a HESI membership fee and a committee activity fee (see paragraph "Anticipated costs for participants" below, for details).

Anticipated Costs for Participants:

- Each participant will be making the contributions to run the assays at their facilities (lab supplies and man hours; estimated at to be between US$10,000 to $20,000 for each assay, depending on the assay). Specific equipment needs as well as estimated costs per methodology are indicated in table 1, based on the experience from MEASURE preliminary studies conducted in Japan.
- For organizations from the private sector new to HESI:
  - HESI membership fee (http://hesiglobal.org/partner/join-from-the-private- sector).
  - CT-TRACS committee fee (re-assessed each year based on committee activities' needs; covers direct expenses to execute committee activities and HESI staff support).

Benefits for participants:

- **Knowledge and know-how:** access preliminary data and protocols from the MEASURE studies; learn from a multi-disciplinary team of experts.
- **Data access:** access new data generated at all sites through this international study (i.e., get the results of 3 studies for the cost of 1).
- **Leadership:**
  - Contribute to the study design with your expertise
  - Help guide the evolution to a more data-driven characterization framework, rather than theoretical.
  - Help lead the development of new standardized approaches for CTPs safety evaluation.

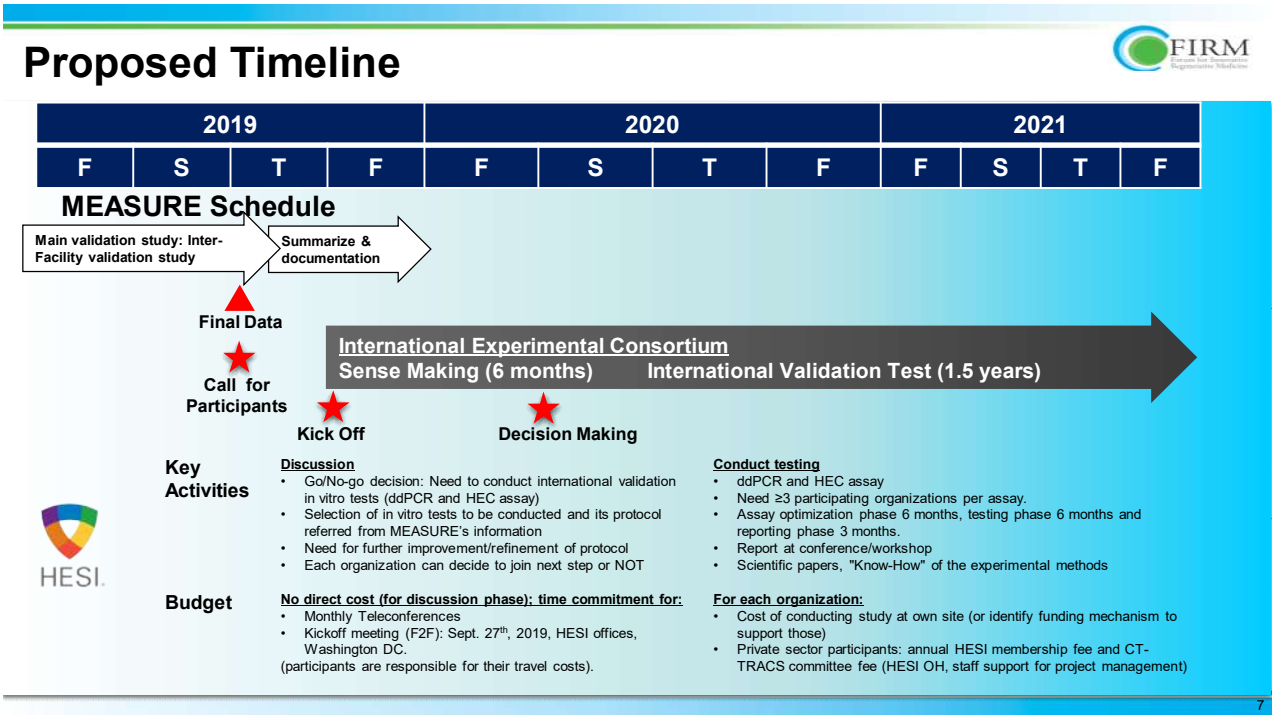
- **Authorship:** publication opportunities and fostering open scientific interaction in the public domain.
- Opportunity to benefit from other CT-TRACS working groups' activities at no extra cost.

Table 1: Specific equipment needs and estimated costs per methodology

Test	Special equipment	Expected cost for testing
PCR-Based Detection	Droplet Digital PCR [Model used for MEASURE: QX200, BioRad (*)]	When contracted out to a CRO: ~ USD 10K. When conducted in-house: cost of PCR reagents/routine lab materials and man hours; (initial set up is USD 170K with equipment).
Highly Efficient Culture	Standard equipment for cell culture; no other special equipment needed.	~ USD 20K plus cost for iPS cells

(*) http://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system?ID=MPOQQE4VY
http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6311.pdf

TENTATIVE TIMELINE:



References:
Herberts et al. 2011. *Journal of Translational Medicine.* 9:29. http://www.translational-medicine.com/content/9/1/29
Basu et al. 2015. *Toxicol Pathol.* 43: 115-25. doi: 10.1177/0192623314559104.
Bailey, 2012. *Sci Transl Med.* 4: 147fs28. doi: 10.1126/scitranslmed.3003685.

Attached*:* Appendix 1 (LOI form) and 2 (*in vitro* assays description).

About CT-TRACS:
CT-TRACS is an international, multi-sector and multi-disciplinary team of experts with interest in sharing their knowledge, discussing common challenges and seeking consensus on harmonized solutions for the safe translation of cellular therapies (a PDF Fact sheet about the committee can be found in the HESI CT-TRACS public website: http://hesiglobal.org/cell-therapy-tracking-circulation-safety-ct-tracs/).
The CT-TRACS "Tumorigenicity WG" was established with the aims to:

- Evaluate the translational utility, reliability and predictive value of existing tools and technologies for assessing the tumorigenicity potential of cell-based therapies, with the ultimate goal to improve their safe application into the clinic.
- Understand current regulatory expectations in different countries; define best practices to build confidence in making safety assessment decisions for tumorigenicity, internationally.

About HESI:
HESI is an independent non-profit dedicated to bringing together global teams of scientists from academia, government, industry, and NGOs to solve the most pressing risk and safety challenges facing humans and the environment today. The research facilitated by HESI's technical committees is designed to identify and test solutions that can be broadly applied. Some of the practical applications of HESI-directed research include improving patient safety, reducing the use of animals in testing, protecting the environment, and enhancing product safety. HESI is based in Washington D.C., USA, but operates globally.

HESI Vision: Creating science-based solutions for a sustainable, healthier world.
Our vision is to:

- Provide regulators/decision bodies with sound science for better, more informed decisions.
- Be the recognized leader in bringing together multidisciplinary teams to solve the tough scientific challenges around risk assessment and safety.
- Encourage collaboration across academia, government, industry, and NGO scientists to protect human health and the environment.
- Create and test technology platforms and scientific frameworks that can be used to more effectively predict the effects on humans or the environment.

Science is advancing at a rapid pace, and we learn more every day about safety and risk. It can be difficult, if not impossible, for the scientific community to keep up with the latest technological

advancements. **HESI strives to help scientists discover, create, and embrace new technologies, methods, and scientific advancements in the areas of risk and safety assessment.**

- HESI helps vet scientific advancements to help increase their adoption as part of a research protocol.
- By bringing together scientists from academia, government, industry, and NGOs at the start of a research project, HESI helps frame what questions need to be asked to make the research applicable and valuable.
- By improving communication across sectors, HESI increases transparency and reduces redundancy.
- **HESI committees offer data-driven solutions:** they moderate conversations between scientists from academia, government, industry, and NGOs to create and test potential technology platforms and scientific frameworks that can be used to more effectively predict the effects on humans or the environment.

Appendix 1 – Application form template (LOI)

Please insert your official letterhead

Title, First Name, Last Name
Position
Institution, Dept.
Street
Zip Code
City
Contact information: email; phone number.

Letter of Intent
for the
"HESI CT-TRACS International Multi-Site Study on *In vitro* Methods to Assess the Tumorigenicity of Human Cell-Based Therapeutic Products"

Submitted as a part of the HESI CT-TRACS call for participants of 06/30/2019 posted on HESI CT-TRACS web-page: https://hesiglobal.org/cell-therapy-tracking-circulation-safety-ct-tracs/.

I the undersigned, confirm on behalf of my organisation, _____ (please insert the name of your organisation) our interest in the above-mentioned project. We believe that the project is in line with the goals and aspirations of our organization.
We therefore fully support this initiative.

By the present LOI, I express commitment to:

- Join and participate actively in the discussion phase of this project (starting September 2019)
- Consider joining the effort as a full participant and provide a final decision to HESI by November 1st, 2019.

I have read CT-TRACS' "Call for Participants" and understand the candidates' eligibility and requirements conditions, as well as the related "anticipated costs for participants", should I confirm my full participation in the study.

This document has not a legally binding character.

_____
Date, full name and signature of the representative.

Appendix 2 – *In vitro* Assays description[1]

I. Assays for detection of residual undifferentiated iPS cells

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

*: eg. cultured on laminin-521 in Essential 8 medium

[1] Description of assays as conducted in the MEASURE study, as starting point for Phase 1. Actual conditions for the International Experimental Consortium will be discussed in Phase 1 and determined by participants. Participants are welcomed to make suggestions for change by presenting the rationale behind and supporting evidence".

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