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CALL FOR PARTICIPANTS: International Collaborative Study for the Identification of Off-Target Mutations Associated with Genome Editing

The Health and Environmental Sciences Institute's Cell Therapy – TRAcking, Circulation & Safety (HESI CT-TRACS) Committee is seeking additional partners (academic/research institutions; government agencies; non-profit institutions; private sector companies or CROs) for an international collaborative project focused on advancing the application of sequencing technologies for identification and evaluation of off-target mutations that may be associated with genome editing via CRISPR Cas9.

This project will be conducted under the auspices of the non-profit HESI in alignment with its mission to provide public benefit by enhancing the science around human health and safety evaluation. This initiative aims to advance the implementation of novel cell and gene therapies by improving the science underlying their safety evaluation.

Study Objectives

Project-1 (Detection with Advanced Sequencing Technologies): The objective of this study is to assess the potential of novel sequencing technologies (e.g., INDUCE-Seq) to identify DNA damage and mutations at CRISPR off-target sites. Induce-Seq is proposed as a means to identify DNA damage *in situ*, without the need for post-generation PCR amplification during sequencing library preparation thus eliminating break sequence amplification bias and making it possible to quantitatively map endogenously produced off-target breaks in the genome. The study also proposes the use of Duplex-Seq to establish whether the DNA damage identified by INDUCE-Seq has further mutational consequences. It is anticipated that analyzing breaks on a genome-wide scale (INDUCE-Seq) coupled with subsequent assessment of the mutational outcome (Duplex-Seq) could help determine the mechanisms that underpin the formation of mutations caused by double-strand breaks (DSBs). This work will be the first collaborative study to explore the feasibility and outcome of combining these technologies for therapeutic safety evaluation and will add to our understanding of the frequency and location of CRISPR Cas9 off target effects.

Project-2 (Functional Assay): once DNA breaks have been identified and a targeted analysis confirmed a potential mutational outcome, a functional assay is needed to understand the consequences of the mutations and translatability of risk. This project aims to evaluate *in vitro* transformation assays for adherent genome edited cells: Soft Agar Colony Forming Assay (SACF), and Growth In Low Attachment (GILA) assay. Current collaborators have developed a digital soft agar assay based on the use of MCF10A (an immortalized, not transformed human mammary epithelial cell line) to assess the tumorigenic potential of CRISPR/Cas9 editing. Preliminary results (Lemmens *et al.,* in preparation; conference presentation materials available upon demand) indicate that this *in vitro* system is suitable and has many advantages over common *in vivo* tumorigenicity studies. The objectives of this project are: (1) to validate this assay in a multisite study and to establish its LOD; (2) to compare the performance of the digital soft agar assay with the Growth In Low Attachment plates (GILA) assay; (3) to add a second cell line (e.g., NeHepLXHT) to broaden the use of this assay and increase his predictive power. This method would be applicable to all genome editing tools used for gene therapy (not limited to CRISPR/Cas9, the system could be modified for TALENs and ZINC fingers).

Although the study objectives have been defined in both projects, a final protocol has not yet been prepared and will be developed by the participating partners.

Rationale

Given the inherent complexity and heterogenous nature of cell therapy products (CTPs), unique considerations and requirements have emerged along their development path. Careful preclinical assessments and tailored strategies are absolute requirements to mitigate potential risks and facilitate their translation to the clinics. Among the key safety concerns, the risk of tumorigenicity has been a central debate in recent years (Herberts et al., 2011; Bailey, 2012; Basu et al., 2015; Sato et al. 2019). 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 collaborative initiative to evaluate methods is critically important not only for product developers but also for regulatory authorities and patients. To our knowledge, there is no similar initiative at the international level at this time.

Role for Partners

The HESI CT-TRACS committee is seeking partners to engage in the study above as part of an international public-private experimental consortium aimed at evaluating *in vitro* testing methods to address tumorigenic potential of various cell therapy products [CAR-Ts; CRISPR/Cas9 gene edited cells; methods for identification of acquired mutation during human pluripotent stem cells (hPSCs) culturing]. Partners may contribute by running portions of the experimental study, assisting in data analysis, supporting material procurements, providing financial support, and/or providing intellectual input into study design, data analysis, and interpretation.

This program seeks a diverse and balanced participant base with engagement from cell therapy developers, CROs, academic research institutions, government laboratories, clinical medicine, regulatory scientists, and other organizations with capabilities to conduct hands-on testing of the proposed methodologies and/or to contribute to study interpretation and evaluation. Participants will engage in the monthly working group calls and will be interacting with HESI and all other sites involved for the duration of the project. Data specific to the project will be centrally collected by HESI and shared amongst all participants via CT-TRACS password protected SharePoint site.

Throughout the study, HESI CT-TRACS staff will provide the infrastructure for the efforts to take place (e.g. scientific program management and strategy, regular teleconferences among participants, coordination, data sharing, and dissemination of the results) and a neutral platform where everyone can interact.

Requirements for Participation:

1. **Public Sector Scientists (academic, government, clinical, NGO).** There are no fees associated with participation in this program. Participants should complete the letter of interest as described below and indicate how they would like to contribute their expertise and/or research facilities. Participation in this project must be self-funded and there are no guarantees of supportive funding from HESI or CT-TRACS to participating public sector members.
2. **Private Sector Scientists (pharma, biotech, CRO, etc.).** Scientists from private sector must join (or continue participation in) the CT-TRACS Committee (and HESI) for the duration of the Project. *See HESI's "Partner With Us" web page: https://hesiglobal.org/partner/partner-with-us/ for more details on fees.*

Anticipated Deliverables:
New assay development; "Know-How" of the experimental methods and best practices; joint publication of scientific articles in peer-reviewed journals.

Project start date:
Start date will depend upon level of interest, but is targeted for second or third quarter of 2021.

Benefits for participants:

- **Be part of the solution / generate new data:** Contribute to greater (and more robust) data availability – a key need for the translation of cell & gene therapies.
- **Knowledge and know-how:** enhance technical capability of researchers via expertise sharing; exchange knowledge and experiences with a multi-disciplinary team of experts.
- **International, Multidisciplinary and Multisector exposure** – the CT-TRACS committee includes participation from government agencies, regulators, academics and industry and benefits from the broader HESI network. See for example authors of CT-TRACS Position Paper on Tumorigenicity by Sato et al., (all CT-TRACS members): bringing stakeholders together from 5 countries, 11 organizations, including the regulatory/gov. agencies: MHRA, MEB and NIHS.
- **Leadership:**
  - Contribute to the study design with your expertise
  - Help guide the evolution to a more data-driven characterization framework, rather than theoretical; increase the relevance and quality of assays.
  - Help the scientific community around the world to understand the tumorigenic potential of the cell & gene therapy products.
- **Authorship:** Participants will jointly publish a summary of the results of the project in the peer-reviewed literature as a committee publication. All participants will have opportunity for authorship. We also anticipate opportunities for presentation at conferences and/or as part of HESI workshops. HESI supports open access publication, in alignment with its efforts to make scientific information available in the public domain.
- **No commercial interest.** HESI's initiatives seek to benefit the public by furthering the development of novel methodologies, improvement of safety science and overall scientific understanding.
- **Drive change:** develop methodologies that can de-risk cell therapies. Help generate the data to support the development of needed harmonized approaches for CTPs safety evaluation, together with key stakeholders.

Contact: Dr. Lucilia Mouriès, CT-TRACS' Senior Scientific Program Manager, lmouries@hesiglobal.org

To express your interest: please submit a **Letter of Interest** accompanied by your **resume** to Lucilia Mouriès (lmouries@hesiglobal.org).

The letter of interest should include: the reason of your interest; how you/your organization expertise relates to the project (e.g., current or past experience developing/using similar methods; tumorigenicity assessment experience); and the type of support you anticipate your team/organization will be able to provide to the study.

Key References:

CT-TRACS position paper: *"Tumorigenicity assessment of cell therapy products: The need for global consensus and points to consider"* Sato et al. 2019. *Cytotherapy*, 21: 1095-1111.

Bailey, 2012. *Sci Transl Med.* 4: 147fs28. doi: 10.1126/scitranslmed.3003685.

Basu et al. 2015. *Toxicol Pathol.* 43: 115-25. doi: 10.1177/0192623314559104.

Herberts et al. 2011. *Journal of Translational Medicine.* 9: 29. https://doi.org/10.1186/1479-5876-9-29

About CT-TRACS (website for more info)
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. 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 (website for more info):
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 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.

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