INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

ISSCR MEMBERS PROVIDE RECOMMENDATIONS DURING FDA LIAISON MEETING

PREPARED BY: SALIHA MOORE, JD, MA AND DEBRA WEBSTER, PHD

8 February 2023

ISSCR held its third FDA Liaison Meeting with the FDA Center for Biologics Evaluation and Research (CBER), Division of Cellular and Gene Therapies (DCGT), Office of Tissues and Advanced Therapies (OTAT) in December 2022

The ISSCR held its annual meeting with FDA's Office of Tissues and Advanced Therapies (OTAT) on December 13, 2022. A main priority of ISSCR's Regulatory Advocacy is to give its members a voice to help educate policymakers about scientific findings and considerations that will help regulators make scientifically informed policy decisions and facilitate the development of advanced stem cell-based therapies and applications. Members of OTAT heard recommendations from a group of ISSCR members and affiliates, including Melissa Carpenter, Chair of ISSCR's Manufacturing, Clinical Translation, and Industry Committee, regarding recommendations on significant topics in the stem cell research field, including:

- Current Status of Prion Diseases and Methods for Prion Detection in Biological Samples
- Reducing Genomic Risk in Pluripotent Stem Cell Therapeutics
- Considerations for Hypoimmune Stem Cell-Derived Therapies

Recap of 2021 ISSCR FDA Liaison Meeting

Melissa Carpenter offered opening remarks, providing a brief background on the ISSCR and its mission as a global organization working to promote excellence in stem cell research and its applications to human health. Following this, Debra Webster provided a recap of the 2021 ISSCR Liaison Meeting. The 2022 meeting built on several topics and recommendations discussed during the 2021 meeting which included:

- International Divergence of Donor Screening and Testing Requirements: Recommendations for Granting Exemptions and Labeling Products under Section 1271.155
- Potential Strategies for Mitigating the Risk from Prion Diseases
- Recommendations for the Use of Genomic Sequencing Data in the Evaluation of Cellular Therapies
- Recommendations for Gene Editing and iPSC Starting Materials
- Considerations for the Use of Sham Surgical Comparators in Clinical Trials Involving Stem Cell-Based Therapies
- Recommendations for Assaying Tumorgenicity of iPSC Therapies

Current Status of Prion Diseases and Methods for Prion Detection in Biological Samples
Presenter: **Claudio Soto, PhD, McGovern Medical School, UTHealth Houston**
Moderator: **Debra Webster, PhD, BlueRock Therapeutics**

This presentation revisited aspects of the 2021 discussion regarding prion disease risks and considered how such risks could be mitigated through development and validation of a prion test implemented for donor-screening, raw material, and product testing. Dr. Soto explained the mechanisms of prion diseases, highlighting that donor screening in high-risk countries only partially addresses the concern with controlling its transmission. In humans, there are different origins of prion diseases such as sporadic, iatrogenic, genetic, and infectious, which have been demonstrated to be transmissible through use of biological, medical, and food products. Thus, the need for validated methods to screen for prion contamination in donors is significant for development of final stem cell therapeutic applications because the administration of contaminated biological products has been the origin of several hundred cases of iatrogenic transmission of reported prion diseases.

Dr. Soto described the amplification of PrPSc during disease propagation over a slow pathological progression which causes severe brain deterioration – potentially decades for humans. PMCA (also known as RT-QuIC) mimics the propagation progressions of prion diseases, allowing for the detection of small amounts of prion particles in blood, urine, and biological products. PMCA has shown sensitive and specific detection of prions in blood and urine of patients affected by vCJD, even at preclinical stages of the disease. Prions can be detected in various other tissues and biological fluids as well as in samples used for production of biological products (stem cells, plasma, urine, etc.), indicating that the technology may have broad application to increase safety of biological products.

ISSCR recommendations for the use of PMCA technology:

- PMCA technology can serve to support donor screening for adventitious agent testing for prions.
- PMCA technology can be an accepted assay to assess the presence of prions in cell banks.
- In this context such an assay should be acceptable as part of a risk-based approach to assess cells derived from donors that have had some risk of TSE exposure or incomplete donor screening.

FDA Responses:
During a brief discussion following Dr. Soto's presentation, Dr. Steven Oh shared that FDA is aware of PMCA testing and recognizes its value. It was indicated that many reasons support FDA's consideration of further developing this technology for use in assessing cell therapies. Dr. Oh recommended a focused discussion with OTAT about further developing this tech, validating methods, and providing this information to FDA and other sponsors.

**Dr. Steven Oh, Chief of Cell Therapies Branch (CTB), OTAT, CBER, FDA*

Reducing Genomic Risk in Pluripotent Stem Cell Therapeutics
Presenter: **Chuck Murray, MD, PhD, University of Washington Institute for Stem Cell and Regenerative Medicine**
Moderator: **Jane Lebkowski, PhD, Regenerative Patch Technologies**

ISSCR's second presentation addressed genomic heterogeneity in pluripotent stem cell therapeutics. Dr. Murry provided an overview of cancer genetics, tools used for genomic analysis, and sources of genomic heterogeneity. It is a priority to mitigate risk at the genome level in translating stem cell applications to clinical therapies. As described, there are 6 orders of magnitude available for use in multiscale genome assessments, and, while this presentation primarily focused on cancer genetics, these techniques can be applied to different areas of genomic risk. Varying sources of genomic heterogeneity including iPSC mutations and dominant clones in PSC cultures pose significant challenges to personalized cancer medicine.

Dr. Murry outlined a recent case study of BCOR mutations in human iPSCs and provided two examples of analytical genomics during stem cell manufacturing, including an analytical workflow were provided. Dr. Murry described the challenge of evaluation genomic heterogeneity as inevitable and that while our ability to detect and quantify mutations is growing rapidly our ability to discern functional consequences is not keeping pace. There is a need to establish which mutations are of concern and what level of allelic frequency is acceptable.

ISSCR recommendations for genomic heterogeneity:

- Gain-of-function mutations in oncogenes and chromosome-level aneuploidies should be avoided, however, smaller-scale copy number variations should be decided on case-by-case bases.
- Because only some tumor suppressor genes are currently understood well enough to disqualify a line if mutated (e.g., TP53), investigators/sponsors should interrogate the genome to identify variants of concern, which should be used to select donors and prioritize clones. Identification can be made drawing from clinical testing as well as clinical and research databases.
- As mosaicism/heterogeneity is inevitable within any cell population, the frequency of undesired variants should be assessed and minimized during process development. There is currently no established threshold between a safe and an unsafe frequency for an undesirable variant, thus, sponsors should monitor and correlate frequencies with toxicology studies and clinical outcomes.
- For the community: Lessons from preclinical and clinical outcomes should be shared with the therapeutic community to enhance patient safety and increase probability of success for the whole field.

FDA Responses:
In response to the proposed discussion topics, Dr. Debra Hursh expressed that FDA has been concerned about p53 mutations for some time and recognizes that BCOR may also be significant. Hursch recommended that sponsors perform whole-exome sequencing

(WES) and use COSMIC or other oncogene database as a reference for variants of concern.

Dr. Anna Kwilas, discussed genome editing, noting that recommendations for genomic analysis by karyotype, aCGH, commercial testing, and exome sequencing are consistent with FDA's recommendations to sponsors. Additionally, analysis should be done throughout the manufacturing process. While the Agency does not expect the cells to be absent of mutations, or to lose mutations present in parental PSCs, it is necessary evaluate whether mutations are occurring during process development. For gene-edited cells the Agency recommends whole-genome sequencing (WGS) of the edited cell banks. If a sponsor notes a mutation of concern in their cell banks, they can perform RNAseq analysis and expression profiling to help understand whether this mutation is significant. Depending on whether and which mutation is expressed in the final product, sponsors may need to perform a risk assessment. The Agency may be concerned about mutations in exons, but it will evaluate the data that has been generated and determine what is acceptable based on the final product.

**Debra Hursh, PhD, Senior Investigator and Product/Chemistry, Manufacturing, and Controls (CMC) Reviewer, Division of Cellular and Gene Therapies, OTAT, CBER, FDA*
**Anna Kwilas, PhD, Gene Therapy Product/Chemistry, Manufacturing, and Controls (CMC)Team Lead, OTAT, FDA*

Considerations for Hypoimmune Stem Cell-Derived Therapies
Presenter: **Deepta Bhattacharya, PhD, Professor of Immunobiology, University of Arizona**
Moderator: **Sonja Schrepfer, MD, PhD, Senior Vice President, Sana Biotechnology; Professor of Surgery, University of California San Francisco**

The final presentation centered on proposed risk-based assessments of hypoimmune cells and strategies to minimize potential of tumor development. Dr. Bhattacharya began his discussion by describing the underlying science of hypoimmune cells, giving detail regarding immune recognition pathways, approaches to creating hypoimmune cells, and assessment of hypoimmune properties of clinical iPSC products. He explained several mechanisms appropriate for assessing hypoimmune properties of clinical iPSC products such as: phenotypic assessment of edited targets; loss/gain of target proteins; T-cell proliferation target killing; antibody and complement deposition; natural killer cell activation; and phagocytosis assays.

As a method sufficient to demonstrate the safety of hypoimmune cells, Dr. Bhattacharya shared a risk-based approach to assessing of hypoimmune cells for minimizing tumor risks. Dr. Bhattacharya suggested 1) characterization of editing reagents; 2) genomic characterization of the master cell bank and final product, and 3) rodent transplantation assays to assess tumorigenicity.

ISSCR Recommendations for Hypoimmune Pluripotent Stem Cell-Based Products:

- Edited iPSC master cell banks and drug substances should be screened for known oncogenic mutations, chromosomal aberrations, and 'extra' DNA integrations in genome.
- When iPSC-derived cell therapies are genetically modified for hypoimmunity, investigators/sponsors should:
    - include evidence of hypoimmune status in cell product characterizations demonstrating cell phenotypes;
    - perform in vitro studies demonstrating hypoimmune characteristics; and
    - perform in vivo GLP studies to demonstrate safety and persistence (e.g. tumor, toxicology, biodistribution).
- The need for additional editing to include a kill or safety switch should be considered product/indication specific rather than a broad requirement.
- Research grade reagents are suitable for seed bank manufacturing provided sponsors/investigators follow appropriate risk mitigation and laboratory controls.
    - Rather than setting a proscribed limit on VCS for risk mitigation, a risk-based approach to VCN should be adopted to evaluate genetically modified iPSC-derived cell therapies.
- There is potential for cells to be infected and serve as a viral reservoir after transplantation, however, adequate knowledge is not available to provide recommendations.

FDA Responses:

FDA does not mandate the use of GMP reagents for single occasion editing early in the manufacturing process, but it requires information about the reagents used including controls and segregation as well as testing methods used to qualify the reagents. Agency representatives suggested that it is best for sponsors to confer with FDA during the pre-IND stage to determine whether the Agency has additional concerns.

ISSCR posed questions regarding whether early PSC banks edited using non-GMP reagents are suitable for Phase 1 clinical trials and later stage clinical development and commercialization. FDA requests detailed information about cell lines and corresponding cell products tested in Phase 1 trials specifically to evaluate non-GMP reagents' suitability. If the trial outcome is promising, the cell banks are likely be a material that can be used for commercialization. However, if new banks must be manufactured, the sponsor should establish stability and comparability between the banks.

Regarding gene editing using lentiviruses and the VCN<5 precedent for in vivo genome editing therapies, the Agency pointed out there is some flexibility. However, while VCN<5 is not a "hard limit" and a higher number can be justified, FDA prefers to keep VCN to a minimum. It is necessary to know where the vectors are inserted, which helps mitigate risk. With new technologies emerging the FDA is seeing higher instances of new edits overlapping with previous edits, thus, it is important for sponsors to be thoughtful in making each modification. Continuously adding edits can increase chances of inversions/translocations and other elements of genomic instability. The Agency

highlighted that it is valuable to look for potential downstream interactions of the edits and that, for qualifying reagents, traceability is critical.

ISSCR Delegation:
Melissa Carpenter, PhD, Chief Scientific Officer of Regenerative Medicine, ElevateBio
Deepta Bhattacharya, PhD, Department of Immunobiology, University of Arizona College of Medicine
Nissim Benvenisty, MD, PhD, Professor, Stem Cell Unit, The Hebrew University of Jerusalem
Luis Borges, PhD, Chief Scientific Officer, Century Therapeutics
Pete Coffey, PhD, Professor, Institute of Ophthalmology, University College of London
Akitsu Hotta, PhD, Professor, Center for iPSC Research and Application, Kyoto University
Jane Lebkowski, PhD, President, Regenerative Patch Technologies
Tenneille Ludwig, PhD, Director, WiCell Stem Cell Bank
Charles Murry, MD, PhD, Director, Institute for Stem Cell and Regenerative Medicine, University of Washington
Martin Pera, PhD, Professor, JAX Center for Precision Genetics, The Jackson Laboratory
Sonja Schrepfer, MD, PhD, Head of Hypoimmune Platform, Sana Biotechnology
Claudio Soto, PhD, Director, George and Cynthia W Mitchell Center for Alzheimer's Disease and Other Brain Related Illnesses, McGovern Medical School, UTHealth Houston
Rajesh Rao, MD, Professor, Center for RNA Biomedicine, University of Michigan
Debra Webster, PhD, Vice President, Regulatory Affairs, BlueRock Therapeutics

ISSCR Staff:
Chris Barry, PhD, Senior Manager, Scientific Industry & Affairs
Tyler Lamb, JD, Director of Policy
Saliha Moore, JD, MA, Manager of Policy
Jack Mosher, PhD, Scientific Advisor