Stem-cell-derived therapeutics are thought to have great potential in various medical applications, such as treatment of, repair, or regeneration of damaged body components; degenerative, inflammatory, and metabolic diseases; and cancer therapy. Emerging stem-cell-based regenerative therapeutics include the following categories:

- human embryonic stem cells (hESCs), which are derived from blastocysts
- adult or somatic stem cells, including mesenchymal stem/stromal cells (MSCs)
- hematopoietic stem/progenitor cells (HSCs)
- tissue-specific progenitor cells (TSPCs) with cell differentiation and tissue repair potentials restricted to certain tissues
- induced pluripotent stem cells (iPSCs), which are produced by reprogramming adult differentiated cells to regain both self-renewing and pluripotent differentiation properties of the stem cell

Prior to their preclinical safety assessment, the stem-cell-derived therapeutics must be manufactured using a validated process and evaluated *in vitro*, using genetic markers and biomarkers, as well as cellular activity assays, if available, for their identity, purity, potency, and genomic stability. Standard design of preclinical studies for the cell types and populations, the animal species and physiological state most relevant to the intended clinical indications and product class, the intended doses, route of administration, and treatment regimen, and the constraint of certain surgical procedures in small animals, the biological and pharmacologic relevance of cell therapeutics-targeted organs to the human counterparts such as the cardiac system, the size of organ, tissue, and blood samples required for assessing multiple endpoints in the same subject, the extended long-term evaluation of tissue regeneration/repair, and safety follow-up.

**Biodistribution and toxicity studies**

The potential traffic/migration of stem cells administered either systemically or locally can pose systemic safety risks in humans. Potential ectopic tissue formation by these cells in non-target tissues or biological systems, and their unintended plasticity and differentiation, may result in undesirable side effects and toxicity. As limited by current methodologies, tissue biodistribution analysis of the cellular therapeutics cannot be performed in human subjects. Therefore, preclinical biodistribution analysis and characterization of stem-cell differentiation patterns are paramount. Differentiation and regenerative/repair functions of stem cells are regulated by their microenvironment (niche). Certain stem-cell types can home to the tissue of origin as well as traffic to distant locations, as exemplified by bone-marrow-derived MSCs recruited to the injury site. In cancer, MSCs have been observed to migrate to metastatic sites and have been implicated in neoplastic activity.

Aside from the ectopic formation-related risks, local non-physiological or toxicity effects should be examined for their relationship to the distributed cells. The presence and persistence of cells at the post-administration intervals are desirable for demonstration of the success of cell delivery and the intended therapeutic duration. In general, a biodistribution study has multiple interval analyses, for example, one week, one month, three months, and six months (terminal). The early time points are to demonstrate the success of delivery and acute biodistribution, whereas the later time points allow the assessment of persistence, chronic distribution, traffic, migration, and biological state of cells. Technically, biodistribution of the therapeutics in target and major filtration organs/tissues and blood is evaluated by quantitative biodistribution analysis using suitable models. Diseased animal models used in the proof-of-concept efficacy studies at the discovery stage would be preferred, although the availability of such models and their compatibility with the safety studies that have a different set of biological endpoints can be limiting factors. Depending on the scope of safety assessment, small animals can be considered in biodistribution, toxicity, and tumorigenicity studies.

On the other hand, large animal models should be used, due to the following factors:

- the constraint of certain surgical procedures in small animals
- the biological and pharmacologic relevance of cell therapeutics-targeted organs to the human counterparts such as the cardiac system
- the size of organ, tissue, and blood samples required for assessing multiple endpoints in the same subject
- the extended long-term evaluation of tissue regeneration/repair
- safety follow-up

Therefore, careful scientific justification is required for the selection of animal species and strains. To overcome the host immune rejection, genetically immunocompromised and/or agent-immunosuppressed animals will be among the models of choice to make the evaluation of cell survival, fate, functionality, and tumorigenicity feasible. The specific endpoints of safety evaluation are the primary determinants in the choice of the models, as the complete understanding of the mechanism of action is not a regulatory requirement.
polymerase chain reaction (qPCR) analysis of gDNA using human-specific gene primers/probe for human specificity, by quantitation, by immunostaining tissue slides using antibodies against human-specific cell markers, and by proliferation and differentiation-specific markers for characterizing their cell type, proliferation and differentiation states, and function.

The function of cells can also be evaluated by staining tissues and subcellular structures at injection sites using conventional chemical dyes such as H&E and any other specialty dyes. For HSCs, flow cytometry may be used for characterization using human-specific antibodies. In a biodistribution study, the toxicity assessment is often integrated as cohorts and includes monitoring health status, body weight, food consumption, serum biochemistry and hematologic profiles, and macroscopic examination of all organs and tissues at necropsy. Injection sites, target and key filtration organs, lesions, and tumor masses with regional lymph nodes must be examined microscopically for histopathological changes. Lesions and tumor masses are subjected to causal assessment by immunostaining for cell therapeutics. If surgical implantation at a sensitive organ site such as the spinal cord is the route of cell administration, special biological tests such as neurobehavioral observation and allostynia assays should be performed to determine the biological consequence with causal assessment.

### Tumorigenicity studies

The risk of tumor formation may vary, depending on the origin of stem cells, the extent of in vitro manipulation, and the administration route and sites. This risk should also be evaluated in relationship to the lineage pluripotency and commitment and the differentiation stage of the intended cell types. For example, it is likely that pluripotent stem cells, including hESCs and iPSCs, have higher risks in tumorigenicity compared to somatic stem cells, such as MSCs and HSCs, whereas hESCs and iPSCs are predicted to have intrinsic properties of teratoma formation. The most sensitive and appropriate model should be selected for conducting tumorigenicity studies. The biological properties, conditions of in vitro manipulation, persistence of cells, route of administration, and intended clinical use should be among the determinants in model selection.

To maximize the sensitivity of tumorigenicity detection, mouse strains with severe combined immunodeficiency (SCID) that have multiple deficiencies in immune cell types, including B cells, T cells, and natural killer (NK) cells, are preferred, if their life expectancy and intrinsic tumorigenicity do not interfere with the assessment of cell therapeutics during the study period. Because few immunocompromised rat strains are available, immunosuppressants may be used in strains that retain undesirable immune functions, such as NK cells. The emerging humanized mouse models hold promise for assessing tumorigenicity in conditions as close as possible to human immune conditions. In tumorigenicity studies, toxicity studies may be integrated in cohorts, as appropriate, in conjunction with biodistribution studies. For hESC-derived cell therapeutics, the assessment of the risk of potentially contaminated hESCs that are known to form teratomas is crucial. A pilot tumorigenicity study is often required to assess the test system for its permissiveness in formation of teratomas from the parental hESCs and sensitivity in teratoma detection. Such studies consist of spiking hESCs into the cell therapeutics at different ratios from 0 to 10%, using 100% parental hESCs as a positive control and, subsequently, implementing the appropriate ratios in the main study. Depending on the persistence of cells, a tumorigenicity study comprises several interval analyses, e.g., three months, six months, and nine months or longer depending on the types and the expected intrinsic properties of stem cell-derived therapeutics and the intrinsic tumorigenicity and life expectancy of immunocompromised animal models. The analysis for causal assessment consists of

- monitoring health status and body weight
- macroscopic and microscopic examination of injection sites and all organs and tissues for lesions and tumor masses
- qPCR and immunostaining analysis of injection sites and target organs for the presence of therapeutic cells
- immunostaining of lesions and masses with lymph nodes, using antibodies against human-specific cell markers and cell state (proliferation/differentiation/function) biomarkers

A final recommendation: discuss the preclinical safety assessment plan with representatives from Center for Biologics Evaluation and Research (CBER) before initiating the study.

Contact Dr. Xiao (jia-hao.xiao@mpi-research.com) to further discuss your in vivo preclinical safety assessment regenerative therapy needs.

### References