The potential for tumorigenesis associated with stem cell treatments represents one of the most pressing safety concerns for both clinicians and patients [1]. Given the often-fragile nature of patients and the profoundly important areas of the body involved, the development of even the smallest of tumors can easily prove problematic. Additionally, the worrying but increasing current trend for patients undergoing unregulated stem cell therapies has further heightened safety-based concerns [2]. These fears have resulted in the exploration of strategies to minimize the risk of tumorigenesis associated with the transplantation of human pluripotent stem cell (hPSC)-derivatives or adult stem cells into the patient. With regard to hPSC-derivied therapies, approaches have generally focused on the identification and removal of potentially problematic cells pre-transplantation, a strategy that currently suffers from low efficiency, high costs, and the potential impairment of cell survival, engrafment, and function [3]. However, post-transplantation strategies to impede tumor growth, such as gene-directed enzyme prodrug therapy (better known as suicide gene therapy) or radiation therapy, may represent a more efficient way forward, even taking into account their own drawbacks. In our first Featured Article from Stem Cells Translational Medicine, Kojima et al. report on a means to selectively ablate potentially tumor-forming cells present following human induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs) transplantation, a strategy used as a treatment for spinal cord injury (SCI) [4]. In a Related Article from Stem Cells, Lee et al. describe the use of external beam radiation therapy (EBRT) as an effective approach to reduce the risk of teratoma formation stemming from the presence of residual undifferentiated cells following the transplantation of hPSC-derived cells [5].

The discovery of an efficient cryopreservation protocol to store and transport stem cells and their derivatives while preserving functionality represents another pressing concern regarding the clinical application of stem cell therapies and also a potentially exciting approach to improve the efficiency and comparability of large-scale stem cell experiments. While hPSCs generally display high sensitivity to freezing and thawing, which induces spontaneous differentiation, low reattachment, and low recovery [6], studies have established that MSCs also lose functionality during these processes. Current cryopreservation protocols involve freezing cells with slow-cooling rates, which causes cryoinjury, and the suspension of cells in cryopreservants, which display varying levels of toxicity [7]. Furthermore, subsequent thawing in conditions of osmotic imbalance and/or the loss of cell-to-cell contact promotes further cell loss. Overall, these processes expose stem cells to a highly stressful environment that will undoubtedly influence their final number and future functionality at the clinical or research level, even in the case of enhanced post-thaw culture rescue. Our second Featured Article from Stem Cells Translational Medicine from Kaind et al. establishes ultra-fast cooling by adherent vitrification in the “TWIST” substrate as a novel means to improve the post-thaw applicability of hiPSCs and their neural derivatives when compared with currently used cryopreservation techniques [8]. In a Related Article from Stem Cells, Chinnadurai et al. report that prelicensing of MSCs with interferon gamma (IFNγ) prior to cryopreservation protects them from post-thaw T cell-mediated apoptosis, although this strategy fails to rescue the lost in vivo tropism of MSCs to the lungs [9].

**Featured Articles**

**Suicide Gene System Promotes Safe and Effective Neural Stem/Progenitor Cell Treatment of Spinal Cord Injury Treatment**

The transplantation of stem cells into the injured spine of SCI patients represents a promising means to recover lost motor function; however, the potential for tumorigenesis remains a significant safety concern. In the hope of improving the safety of stem cell therapies for SCI, researchers led by Hideyuki Okano and Masaya Nakamura (Keio University School of Medicine, Tokyo, Japan) assessed the potential of a suicide gene system in an hiPSC-NS/PC line known to undergo tumorigenic transformation [10]. The suicide gene system in question used the lentiviral transduction of hiPSC-NS/PCs with a herpes simplex virus type 1 thymidine kinase (HSVtk) transgene and treatment with the ganciclovir prodrug [11]. The selective expression of the HSVtk transgene in proliferative cells converts ganciclovir into a cytotoxic form that selectively kills the potentially tumorigenic...
proliferative cells while preserving mature post-mitotic neurons. In their new *Stem Cells Translational Medicine* article [4], Kojima et al. demonstrate the successful application of their suicide gene system via the transplantation of transgene-modified hiPSC-NS/PCs into the injured spinal cords of immuno-deficient mice. Encouragingly, the selective ablation of proliferating donor cells via ganciclovir treatment both inhibited tumor growth in the spinal cord and permitted a stem cell-mediated protective effect on motor function. While the use of transgenes and the associated lentiviral transduction required do represent limitations to this strategy [11], the authors still see promise in this approach to the improvement of stem cell therapy safety.

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### Increasing Scalability and Comparability of Stem Cell Research with a Novel Cryopreservation Technique

The widespread clinical application of hiPSCs and their derivatives requires an effective cryopreservation strategy for storage and transport that also preserves cell number and function after thawing. Researchers from the laboratories of Beate Winner (Friedrich-Alexander-Universität, Erlangen-Nürnberg) and Julia C. Neubauer (Fraunhofer Institute for Biomedical Engineering, Germany) knew that the current gold standard for cryopreservation, slow-rate freezing of dissociated colonies in suspension, suffers from low survival rates after thawing, and so sought to develop a new and more efficient cryopreservation technique. In their recent *Stem Cells Translational Medicine* article [8], Kaindl et al. describe the ultrafast cooling by adherent vitrification of healthy and Parkinson disease human iPSCs and small molecule-induced neural precursor cell derivatives with the help of the so-called TWIST substrate—a device combining cultivation, vitrification, storage, and post-thawing cultivation [12]. While traditionally cryopreserved cells displayed evidence of cell death-associated damage to cellular membranes after thawing, adherent vitrification preserved cell membrane viability and cell-cell and cell-matrix adhesions thereby providing for a significant increase in overall post-thaw cell number and viability. Interestingly, immunocytochemical analysis and RNA-sequencing demonstrated a lack of significant alterations to gene and pluripotency marker expression post-thawing, suggesting that cryopreservation of hiPSCs fails to alter the stem cell transcriptome significantly. The authors of this new study establish adherent vitrification as an improved cryopreservation technique for hiPSCs and their derivatives with the potential to improve stem cell therapies and promote greater efficiency and comparability in large-scale stem cell experiments.

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### RELATED ARTICLES

**Proof of Concept for External Beam Radiation to Prevent Pluripotent Stem Cell-Derived Teratoma Formation**

The transplantation of hPSC-derived cells at or near sensitive sites within the body, such as the spinal cord, the brain, or the eye, implies potentially devastating consequences to the patient from the development of even the smallest of tumors from “left-over” undifferentiated cells. With this problem in mind, researchers from the laboratories of Joseph C. Wu and Patricia K. Nguyen (Stanford University School of Medicine, California, USA) assessed the potential of EBRT to inhibit the potential development of teratoma tumors from errant hPSCs [13]. EBRT represents one of the primary modalities used in the oncologic treatment of solid tumors, and Lee et al. report that targeted EBRT promoted the long-term growth-arrest of human embryonic stem cell- and hiPSC-derived teratomas at day 28 post-transplantation in a small animal model and reduced the reseeding potential of teratoma cells during serial transplantation experiments. Overall, the authors of this *Stem Cells* study [5] established that the application of EBRT promotes teratoma cell apoptosis, senescence, growth arrest, and the disruption of the tumor vasculature while limiting damage to the surrounding tissues. These encouraging findings serve as a proof-of-concept for the exploitation of EBRT in the treatment of
Preserving the Function of Cryopreserved Mesenchymal Stem Cells to Increase Therapeutic Efficacy

The freeze–thaw cycles associated with the cryopreservation process required for the widespread application of stem cell therapies and improved standardization of stem cell research can negatively impact stem cell functionality, including the immunosuppressive capabilities of MSCs [14]. Researchers from the laboratory of Jacques Galipeau (Emory University, Atlanta, GA, USA) discovered that cryopreservation and thawing of MSCs significantly inhibited MSC-mediated immunosuppression through high levels of susceptibility to activated T-cell-mediated contact-dependent apoptosis. Interestingly, the study also discovered that allogeneic cells suffered to a greater degree than autologous cells. As an approach to mitigate this problem and boost the immunosuppressive effect of MSCs, the authors assessed a hypothesis that IFN-γ-licensed MSCs may serve to enhance the therapeutic efficacy of MSC in clinical use.

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REFERENCES

1 Duinsbergen D, Salvatori D, Eriksson M et al. Tumors originating from induced pluripotent stem cells and methods for their prevention. Ann N Y Acad Sci 2009;1176:197–204.

2 Berkowitz AL, Miller MB, Mir SA et al. Glioproliferative lesion of the spinal cord as a complication of “stem-cell tourism.” N Engl J Med 2016;375:196–198.

3 Lee AS, Tang C, Rao MS et al. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. Nat Med 2013;19:998–1004.

4 Kojima K, Miyoshi H, Nagoshi N et al. Selective ablation of tumorigenic cells following human induced pluripotent stem cell-derived neural stem/progenitor cell transplantation in spinal cord injury. Stem Cells Translational Medicine 2019;8:260–270.

5 Lee AS, Tang C, Hong WX et al. External beam radiation therapy for the treatment of human pluripotent stem cell-derived teratomas. Stem Cells 2017;35:3994–4000.

6 Reubinoff BE, Pera MF, Vajta G et al. Effective cryopreservation of human embryonic stem cells by the open pulled straw vitrification method. Hum Reprod 2001;16:2187–2194.

7 Cohen RI, Thompson ML, Schryver B et al. Standardized Cryopreservation of Pluripotent Stem Cells. Curr Protoc Stem Cell Biol 2014;28:1C.14.1–1C.14.10.

8 Kaindl J, Meiser I, Major J et al. Zooming in on cryopreservation of hPSCs and neural derivatives: A dual-center study using adherent vitrification. Stem Cells Translational Medicine 2019;8:247–259.

9 Chinnadurai R, Copland IB, Garcia MA et al. Cryopreserved mesenchymal stromal cells are susceptible to T-cell mediated apoptosis which is partly rescued by IFNγ licensing. Stem Cells 2016;34:2429–2442.

10 Iida T, Iwanami A, Sanosaka T et al. Whole-genome DNA methylation analyses revealed epigenetic instability in tumorigenic human iPSC cell-derived neural stem/progenitor cells. Stem Cells 2017;35:1316–1327.

11 Zarogoulidis P, Darwiche K, Sakkas A et al. Suicide gene therapy for cancer—Current strategies. J Genet Syndr Gene Ther 2013;4:16849.

12 Beier AF, Schulz JC, Zimmermann H. Cryopreservation with a twist—Towards a sterile, serum-free surface-based vitrification of hESCs. Cryobiology 2013;66:8–16.
13 Zhou H, Rodriguez M, van den Haak F et al. Development of a micro-computed tomography based image-guided conformal radiotherapy system for small animals. Int J Radiat Oncol Biol Phys 2010;78:297–305.

14 Moll G, Alm JJ, Davies LC et al. Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? Stem Cells 2014;32:2430–2442.

15 Duijvestein M, Wildenberg ME, Welling MM et al. Pretreatment with interferon-γ enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. Stem Cells 2011;29:1549–1558.