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Emerging innovation towards safety in the clinical application of ESCs and iPSCs

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The Review by Behfar and colleagues (Cell therapy for cardiac repair—lessons from clinical trials. *Nat. Rev. Cardiol.* **11**, 232–246; 2014)[1] summarized that 'first-generation' cell therapies for heart failure[2] using autologous cells are safe for use in humans. Conversely, 'next-generation' cell therapies, which include pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have major safety concerns, because contamination of undifferentiated cells might lead to teratoma formation.[3] However, novel and efficient protocols for selective shutdown of tumour formation in these cells have been reported in several studies, which merit discussion (Table 1).

Firstly, chemical inhibitors of survivin potently induce selective and complete cell death of undifferentiated human ESCs or iPSCs.[4,5] A single pretreatment exposure to survivin inhibitors is sufficient to completely inhibit teratoma formation after transplantation.[4] Importantly, differentiated cells derived from human ESCs or iPSCs maintain their functionality after treatment with survivin inhibitors.[4] The survivin inhibitor QC has been widely used as nutritional supplement and no adverse effects have been reported.[4] Secondly, chemical inhibitors of oleate synthesis have been identified as compounds for selective elimination of human ESCs or iPSCs.[6,7] Oleate synthesis inhibitors lead to apoptosis in human ESCs or iPSCs through lipid metabolism, revealing a dependence of these cells on oleate. At present, application of oleate synthesis inhibitors is limited to *in vitro* culture before transplantation; whether these inhibitors might be applied *in vivo* remains to be determined. Thirdly, the diabetes mellitus drug metformin[8] can reduce tumour forming potential of iPSCs without affecting pluripotency;[9] however, in this study only mouse iPSCs were investigated. Metformin, an agonist of AMP-activated protein kinase, suppresses the expression of Oct4 and survivin thereby showing previously unrecognized stem-cell toxicity.[10] Finally, an antibody against stage-specific embryonic antigen-5 (a newly identified PSC-specific surface antigen) can be used to remove undifferentiated cells by fluorescence-activated cell sorting.[11] However, because this method depends on cell sorting, which includes *ex vivo* manipulation (such as single-cell dissociation and cell-staining techniques), cells might lose viability. New synthesized small molecules (such as JC011), which selectively induce a cytotoxic endoplasmic reticulum stress response in ESCs and iPSCs, have also been reported, but further studies should reveal the precise mechanisms of this pathway.[12]

We believe that two issues relating to the use of ESC or iPSC therapies need to be addressed. After treating cells with chemical inhibitors to prevent teratoma, these cells should be tested to ensure that they have maintained functional properties, including differentiation capacity[13] and engraftment potential. Efficiency, as well as safety, is required for ideal cell transplantation. A second problem is that malignant cell transformation, other than teratoma formation, after transplantation of PSC-derived cells might also exist. Pluripotent tumour forming potential can be divided into two categories: malignant transformation of differentiated PSCs, and benign teratoma formation from residual undifferentiated PSCs.[14,15] The former should be also investigated for safety. For example, CD30, which is a biomarker for transformed human ESCs, is correlated with karyotype abnormalities such as partial duplication of chromosome.[16] Further elucidation of this issue is needed before a judgement on iPSC clinical safety can be made.

Table 1 | Novel strategies for selective elimination of teratoma

Reference	Chemical or antibody	Mode of action	Drug
Lee *et al.*[4]	Chemical inhibitor	Survivin inhibition	QC; YM155
Ben-David *et al.*[6]	Chemical inhibitor	Oleate synthesis inhibition	PluriSIn #1
Vazquez-Martin *et al.*[9]	Chemical inhibitor	AMP-activated protein kinase activation	Metformin
Tang *et al.*[11]	Antibody	SSEA-5 purging	Anti-SSEA-5 monoclonal antibody
Richards *et al.*[12]	Chemical	Endoplasmic reticulum stress	JC011

Abbreviations: PluriSIn, pluripotent cell-specific inhibitor; SSEA-5, stage-specific embryonic antigen-5.

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Competing interests
The authors declare no competing interests.

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