

The Need for Genetically Engineering Therapeutic Pluripotent Stem Cells

The advent of induced pluripotent stem (iPS) cell technology, pioneered by Shinya Yamanaka, is poised to have a major impact in biology and medicine. Human iPS cells will probably be useful for disease modeling, drug screening, and, perhaps, cell-based therapies. Although the potential clinical applications are still remote, it is timely to reflect on how iPS cell progeny may be introduced in the clinic. The field of gene therapy has much to offer for the development of iPS cell-based therapies.

Although still uncertain, the therapeutic potential of iPS cells is tantalizing. Human iPS cells can be generated from various cell types, by way of a variety of strategies that utilize integrating and nonintegrating vectors, recombinant proteins, and RNA. These reprogrammed cells often display a variable, and thus far unpredictable, differentiation potential. The degree of epigenetic remodeling, genetic integrity, and tumor-forming ability of individual iPS cells are thus unique and possibly dynamic features that warrant detailed characterization on a clonal basis. A consensus operational definition for human iPS cells has yet to be promulgated.

Although important questions pertaining to the mechanisms governing the efficacy, reproducibility, and outcome of cellular reprogramming remain to be addressed, there have been rapid advances in the derivation of several somatic cell types, including, for example, hepatocytes and dopaminergic neurons. Even though the generation of other cell types, such as hematopoietic stem cells, remains elusive, we can anticipate that various iPS cell-derived cell types of potential therapeutic utility will be available in the next three to four years. Will these emerge as “cell therapies” or “cell and gene therapies”?

iPS cells and genetic engineering have intertwined histories, and they are poised for a long-term relationship. iPS cells were born out of a genetic screen utilizing γ -retroviral vectors to shuffle combinations of candidate reprogramming transcription factors into fibroblasts. Although one of the early points of discussion revolved around the generation of “transgene-free” iPS cells, it is important to realistically assess the risks and benefits of genetically engineered iPS cells in the context of their evaluation in phase I clinical studies.

The manifold safety concerns raised by the use of iPS cell-derived cell products include the risk of teratoma formation from pluripotent cells persisting in bulk differentiation cultures, the risk of reactivating integrating reprogramming vectors (if they were not excised after reprogramming), the risks of insertional mutagenesis, cell culture-induced mutagenesis, and genome editing-induced mutagenesis.

Importantly, while some of these risks are the consequence of genetic manipulations, others are not. The latter are the consequence of biological properties that are inherent to pluripotent cells (i.e., applicable to any “transgene-free” pluripotent cell), the effects of prolonged cell culture, and the unknown fate of cells generated through *in vitro* directed differentiation following prior reprogramming. Considering the intrinsic risks of iPS cells, will the addition of a foreign gene, especially if inserted into a genomic “safe harbor,” add to their baseline risk level? More significantly, will genetic engineering not enhance the clinical investigation and safety of iPS cells?

Genetic engineering steps intersect the clinical development of iPS cells at several levels: for the generation of iPS cells; for gene marking to distinguish infused cells from host cells (which is essential in an autologous setting) and to enable cell tracking or imaging; for safety switches and suicide genes to control or treat teratoma formation or secondary cell transformation; and, of course, for genetic correction modalities—via gene repair or gene addition—used for the treatment of monogenic disorders with autologous cells.

Thus, although transgene-free iPS cells are highly appealing, the use of transgenes will provide major advantages for the functional evaluation and safety of pluripotent stem cells in early clinical studies. Key tasks in this regard are the identification and validation of genomic “safe harbors” as well as devising safe and robust methodologies to target such sites. These efforts and others in the genetic engineering of iPS cells on their path to clinical development warrant further discussion, to which *Molecular Therapy* will contribute through a series of Commentaries and Reviews in upcoming issues.

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