Cell Stem Cell
Forum

Toward the Development of a Global Induced Pluripotent Stem Cell Library

Marc Turner,[1] Stephen Leslie,[2] Nicholas G. Martin,[3] Marc Peschanski,[4] Mahendra Rao,[5] Craig J. Taylor,[6] Alan Trounson,[7] David Turner,[8] Shinya Yamanaka,[9,10] and Ian Wilmut[11,*]
[1]Medical Director, SNBTS HeadQuarters, 21 Ellen's Glen Road, Edinburgh EH17 7QT, UK
[2]Statistical Genetics, Murdoch Childrens Research Institute, and Department of Mathematics and Statistics, The University of Melbourne, Flemington Road, Parkville, Victoria 3052, Australia
[3]Queensland Institute of Medical Research, 300 Herston Road, Brisbane Q4029, Australia
[4]INSERM UMR861, Stem, AFM, 5 rue Henri Desbruères, Evry 91030 cedex France and UEVE UMR861, 5 rue Henri Desbruères Evry 91030 cedex France
[5]NIH Center for Regenerative Medicine, 50 South Drive, Suite 1140, Bethesda, MD 20892, USA
[6]Tissue Typing Laboratory (Box 209), Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, UK
[7]California Institute of Regenerative Medicine, 210 King Street, San Francisco, CA 94107, USA
[8]SNBTS, Royal Infirmary of Edinburgh, EH16 4SA, UK
[9]Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University, Kyoto, 606-8507 Japan
[10]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[11]MRC Centre for Regenerative Medicine, Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh BioQuarter, 5, Little France Drive, Edinburgh, EH16 4UU, UK
*Correspondence: ian.wilmut@ed.ac.uk
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The ability to preselect the donor genotype of iPSC lines provides important opportunities for immune matching in cell therapy. Here we propose that an international assessment should be made of how immune incompatibility can best be managed and how a network of GMP HLA homozygous haplobanks could be operated.

Much has been written about the potential contribution of human pluripotent-stem-cell-line-derived cellular therapeutics to the future realization of regenerative medicine. However, the development of clinical cellular therapeutics from these sources requires attention to issues normally associated with blood, tissue, and organ transplantation, such as donor consent, selection and screening, and immune compatibility, as well as issues related to clinical good manufacturing practice (GMP), quality assurance, and regulatory compliance. A key clinical issue will be the extent to which pluripotent-stem-cell-line-derived cellular therapeutics are likely to be immunologically compatible with patients. The ability to preselect the donor genotype of induced pluripotent stem cell (iPSC) lines provides opportunities for immune matching that were not available with embryo-derived pluripotent cell (hESC) lines (Taylor et al. 2012; Rao et al. 2012). While it is possible that clinical GMP-grade autologous iPSC lines could be derived on an individual basis, it seems unlikely that these will be used as a source for large numbers of patients in the near future, given the time and cost required to produce clinical GMP cell lines and to differentiate these into cells and tissues of clinical utility. It is likely, therefore, that a bank of allogeneic clinical GMP cell lines will be required to allow the field to develop over the next few years, raising the issues of how such a bank, or network of banks, could be established and, in particular, how immune incompatibility can best be managed.

Solid organ transplantation benefits from at least partial HLA matching and systemic immune suppression in order to reduce the incidence and severity of graft rejection. In the case of hematopoietic stem cell transplantation (HSCT), even more stringent HLA matching is required to reduce the risk of both rejection and graft versus host disease (GvHD), though GvHD should not occur with hESC or iPSC-derived cell or tissue transplants due to the lack of donor T cells in the graft. Immune suppression carries long-term side effects and the risks of recurrent infection, some forms of neoplasia, and cardiovascular disease. Despite the fact that pluripotent stem cell lines and cells derived therefrom appear to demonstrate low expression of HLA antigens (Drukker et al. 2006), it is clear that differentiated cells do generate an allogeneic response as seen by T cell proliferation and cellular apoptosis against iPSC-derived tissue-specific cells in mice (Guha et al. 2013). Further evidence of immune reactivity against allogeneic cell therapy targets is provided by the observation that cytotoxic reactivity and alloantibodies can be produced against mesenchymal stromal cells (Schu et al., 2012) and the clinical finding that upon cessation of immunosuppression, HLA antibodies are commonly found in recipients of HLA-mismatched islet transplants (Campbell et al., 2007). Immune T cell activation against human cell, tissue, and organ grafts occurs via both direct and indirect antigen presentation. The direct route involves antigen-presenting cells (APCs) within the graft itself whereas the indirect route requires the processing and presentation of foreign proteins by the recipient's own APCs. It is likely that in cellular therapeutics the indirect route will predominate due to the absence of donor APCs in the cultured cells. However, the indirect route of activation still provides a potent mechanism for the initiation of the alloimmune response, and the immune state itself is dynamic—other stressors can induce rejection at a later stage. In addition, NK cells are also likely to contribute to the rejection of mismatched tissues, particularly single-cell

transplants. NK cell inhibitory receptors (killer cell immunoglobulin-like receptors: KIRs) recognize "self" HLA, but NK cells become activated by the absence of "self" (thus evading T cell recognition) as seen with certain cancers, viruses, and HLA-mismatched allogeneic HSCT. Thus for the production of cellular therapeutics where long-term integration of cells is desired, some degree of HLA matching of allogeneic pluripotent stem cell lines to the potential recipient population is highly desirable. The questions then become, "What degree of HLA matching is necessary, and what is the optimal strategy for the design of a cell bank which could serve these requirements?"

Several levels of immune compatibility need to be considered:

- Carbohydrate blood group antigens such as the ABO system are expressed by all human cells, and individuals who lack expression of the relevant glycosyl-transferases produce naturally occurring anti-A and anti-B isohaemagglutinins, which can cause hyperacute rejection of an incompatible cellular graft.
- HLA incompatibility between donor cells, tissues, or organs and the recipient gives rise to rejection. One would therefore wish to match as many HLA-class I and II loci as is practical in order to minimize this risk and the degree of immunosuppression required. Over 9,000 alleles have been identified within the HLA system, which makes this difficult to achieve, but linkage disequilibrium within the HLA region raises the possibility that individuals are likely to exist that are homozygous for common HLA haplotypes. Such individuals will produce stem cell lines that are HLA compatible with a high proportion of the population.
- It should be borne in mind that even HLA-A, -B, and -DR matched cells, tissues, and organs may be rejected if there are mismatches between the donor and recipient for other HLA loci or minor histocompatibility antigens. Because many of the latter reside on the Y chromosome, it would be preferable to build the HLA haplobank from female donors where possible, although it should be noted that there are potential problems with reactivation of the X chromosome in some iPSC lines.
- Finally, there is evidence that pluripotent stem cells and their derivates can be immunogenic as a result of antigen rearrangements arising from the derivation and culture protocols.

On the basis of first field HLA typing (two-digit typing), Taylor et al. (2012) first estimated that just 10 cell lines homozygous for common HLA types selected from 10,000 individuals could provide an HLA antigen match at HLA-A, HLA-B, and HLA-DR for at least 37.7% of the UK population and a potentially beneficial match for 67.4%. Further work from this group calculated the optimal combination of homozygous HLA types that would match the UK population and identified the 150 most useful homozygous HLA types present among 17 million HLA-typed volunteer stem cell donors registered on Bone Marrow Donors Worldwide that could provide a match for 93% of the UK population (Taylor et al. 2012) when the subject of allele-level matching was also addressed. Nakatsuji et al. have estimated that 30 homozygous cell lines selected from 15,000 individuals would match 82.2% of the Japanese population at the HLA antigen level for HLA-A, HLA-B, and HLA-DR, with 50 homozygous lines selected from 24,000 individuals matching 90.7% of that population (Nakatsuji et al. 2008). Similarly, Okita et al. estimated that, when focusing on higher-resolution HLA-allele-level matching, 140 homozygous lines selected from 160,000 individuals would provide a match for 90% of the Japanese population (Okita et al. 2011). However, these studies in relatively homogenous populations underestimate the challenge in populations with more diverse ancestral and racial backgrounds.

Gourraud et al. (2012) have used a probabilistic model to estimate the number of donors required to construct a haplobank in populations from four different ancestral backgrounds. A bank of 20 homozygous cell lines would require the screening of some 26,000 Northern Europeans and would match more than 50% of that population, but would require the screening of 110,000 African Americans to achieve a match of 22% of that population. A bank of 100 homozygous cell lines from each of these populations would match around 78% of Northern Europeans, 63% of Asians, and 52% of Hispanics, but only 45% of African Americans.

Given the large numbers of individuals who would need to be screened, particularly if gender and blood group are taken into account, we suggest that established cohorts of HLA-typed individuals, such as the more than 22 million people on worldwide hematopoietic stem cell donor registries, cord blood banks, and platelet apheresis panels (Taylor et al. 2012, Rao et al. 2012), would provide a cost-effective way to identify and contact donors who have already consented to donate blood or tissue for clinical purposes and have a high level of traceability. In addition, HLA types can be imputed from genome-wide association studies (GWAS) (Dilthey et al. 2013) that have already been performed for millions of people of most major ethnic groups. It would be helpful if major health foundations could support further GWAS, particularly in regions of high genetic diversity, smaller ethnic groups, and peoples from underdeveloped countries where established HLA-typed clinical panels are less common. Work to establish a blood-derived iPSC bank has been initiated in Japan (S. Yamanaka, personal communication) and is under consideration in the US and Europe.

An international network of mutually recognized iPSC banks would enable the broadest access to this new generation of cellular therapeutics for people of different ancestral and ethnic backgrounds. However, a number of challenges will have to be addressed if this vision is to be realized.

First, informed consent is normally given for direct clinical use of platelet or hematopoietic progenitor cell donations by the donor or, in the case of cord blood, by his/her parents. It is likely that further or more extensive consent will be required for cell donation for iPSC generation given that a permanent cell line will be established and disseminated and may be subject to as yet unforeseen academic, clinical, and commercial developments. Second, since it is possible that a single cell line could be used to generate different cellular therapies that may themselves be used to treat many patients over

a prolonged period of time, there is a risk of amplification of the adverse effects of an infection such as BSE or of genetic abnormality present in the donor. Thought therefore needs to be given to the extent of donor selection and screening. Established, new, and emergent infections will need to be considered along with those agents that may be nonpathogenic in normal individuals but give rise to disease in subsets of patients such as those who are immunosuppressed. Moreover, genetic polymorphism in the donor could conceivably give rise to failure of a cellular therapeutic derived therefrom or disseminated disease in recipients. Third, given that traceability will have to be retained between donor and recipients for many years, if not decades, the extent to which donors should be informed of "abnormalities" discovered in their cell lines—particularly where these may have implications for the health of the donor, his/her family, or the wider public health—should be addressed. Finally, since it is likely that a number of different facilities will be engaged in the manufacture and banking of iPSCs, it is essential that agreement is reached over (inter alia) quality control standards of derivation, characterization, and monitoring of the cell lines themselves, including the use of common nomenclature, identity and potency assays, and reference standards. It is critical that these cell lines are acceptable across the range of international regulatory bodies if cellular therapeutics derived therefrom are to have general clinical applicability.

Given that it is now possible to impute HLA types from GWAS, that international HLA-typed panels, registries, and banks already exist, and that protocols for the derivation of iPSC from such samples have been verified, we suggest that now is the time to start putting together the international collaboration needed to reach agreement on these issues before the methods required for large-scale production of cells at clinical grade are established. We note that several nontherapeutic-grade iPSC banking efforts that will include hundreds or thousands of lines are well underway, indicating that both the will and the infrastructure exist to develop a relatively small registered and quality-controlled panel uniquely dedicated to cell therapy. Such an initiative would enable the global application of this new generation of regenerative therapeutics in a timely and equitable manner.

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