Review

Haplobanking induced pluripotent stem cells for clinical use

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A B S T R A C T

The development of induced pluripotent stem cells (iPSCs) by Shinya Yamanaka and colleagues in 2006 has led to a potential new paradigm in cellular therapeutics, including the possibility of producing patient-specific, disease-specific and immune matched allogeneic cell therapies. One can envisage two routes to immunologically compatible iPSC therapies: using genetic modification to generate a 'universal donor' with reduced expression of Human Leukocyte Antigens (HLA) and other immunological targets or developing a haplobank containing iPSC lines specifically selected to provide HLA matched products to large portions of the population. HLA matched lines can be stored in a designated physical or virtual global bank termed a 'haplobank'. The process of 'iPSC haplobanking' refers to the banking of iPSC cell lines, selected to be homozygous for different HLA haplotypes, from which therapeutic products can be derived and matched immunologically to patient populations.

By matching iPSC and derived products to a patient's HLA class I and II molecules, one would hope to significantly reduce the risk of immune rejection and the use of immunosuppressive medication. Immunosuppressive drugs are used in several conditions (including autoimmune disease and in transplantation procedures) to reduce rejection of infused cells, or transplanted tissue and organs, due to major and minor histocompatibility differences between donor and recipient. Such regimens can lead to immune compromise and pathological consequences such as opportunistic infections or malignancies due to decreased cancer immune surveillance. In this article, we will discuss what is practically involved if one is developing and executing an iPSC haplobanking strategy.

1. Introduction

The development of induced pluripotent stem cells (iPSCs) by Shinya Yamanaka and colleagues in 2006 (Takahashi and Yamanaka, 2006) has led to a potential new paradigm in cellular therapeutics, including the possibility of producing patient-specific, disease-specific and immune-matched allogeneic cell therapies. One can envisage two routes to immunologically-compatible iPSC therapies: using genetic modification to generate a 'universal donor' with reduced expression of Human Leukocyte Antigens (HLA) and other immunological targets (Torikai et al., 2013) or developing a haplobank containing iPSC lines specifically selected to provide HLA matched products to large portions of the population.

HLA matched lines can be stored in a designated physical or virtual global bank termed a 'haplobank'. The process of 'iPSC haplobanking' refers to the banking of iPSC cell lines, selected to be homozygous for different HLA haplotypes, from which therapeutic products can be derived and matched immunologically to patient populations (Riolobos et al., 2013, Gourraud et al., 2012).

By matching iPSC and derived products to a patient's HLA class I and II molecules, one would hope to significantly reduce the risk of immune rejection and the use of immunosuppressive medication. Immunosuppressive drugs are used in several conditions (including autoimmune disease and in transplantation procedures) to reduce rejection of infused cells, or transplanted tissue and organs, due to major and minor histocompatibility differences between donor and recipient. Such regimens can lead to immune compromise and pathological consequences such as opportunistic infections or malignancies due to decreased cancer immune surveillance.

In this article, we will discuss what is practically involved if one is developing and executing an iPSC haplobanking strategy.

2. Rationale for HLA haplobanking iPSCs for clinical use

Clinical-grade iPSC lines are those, through their origin, properties

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and treatment, deemed appropriate starting materials for subsequent human therapeutic development (Wang et al., 2015). Derivation, expansion, characterisation and regulatory approval of clinical-grade iPSC lines is a complex and expensive task. Development of a global HLA haplobank network would drive down cost and maximize patient coverage. For the haplobank network concept to work however, it must operate globally according to the same quality control parameters, assays and standards, so that a line generated in one bank can be accepted and used by others in the network. The Global Alliance for iPSC Therapies (GAiT) is a not for profit organisation which is working with partners involved in clinical-grade iPSC derivation in order to standardise methodologies for quality control as well as to investigate the feasibility of creating a virtual HLA selected haplobank.

3. HLA polymorphism, function and consequences for haplobanking

The human Major Histocompatibility Complex (MHC), the HLA system, is the most polymorphic of all human gene systems and has evolved to deal with a wide range of foreign pathogens and to combat cancer. There are, as of October 2020, over 28,000 recognized alleles (Robinson et al., 2020).

- HLA-A 6,291
- HLA-B 7,562
- HLA-C 6,223
- HLA-DRB 3,536
- HLA-DQB1 1,930
- HLA-DPB1 1,654

The HLA class I (HLA-A, B, C) and class II (HLA-DR, DQ, DP) molecules function to present self and foreign antigens to CD8+ and CD4+ T cells respectively, helping to initiate and sustain adaptive immune responses. The HLA genes, HLA-A, B, C, DRB1/3/4/5, DQB1, DQA1, DPB1 and DPA1 are located on the short arm of chromosome 6 (6p21.3). The allelic variants at each locus are usually inherited as a block, or HLA haplotype (Fig. 1a). HLA haplotypes are inherited from both parents and therefore most individuals will be HLA haplotype heterozygous. The hyper-polymorphism of the HLA genes means that many HLA haplotypes exist in the worldwide population. It should also be noted that frequency of HLA haplotypes varies between populations so that those identified as common in one population may be rare in other populations (Gragert et al., 2013). The concept of haplobanking is that iPSC derived from donors who are homozygous for a particular HLA haplotype will match any patient that carries that haplotype (Fig. 1b). If the haplotype is common in the population, many patients will benefit from this selected donor, but usually the number of common haplotypes in a population is limited. Consequently, to HLA match a reasonable proportion of patients in a population, a relatively high number of HLA haplotype homozygous iPSC lines are required.

Because of the regional variability observed in HLA types, banks set up to cover the highest proportion of patients in one population will not necessarily be of use in other geographical areas, although importantly there will likely be some overlap of useful haplotypes between populations (Pappas et al., 2015). It is for this reason that a global haplobanking collaboration can be envisaged where resources are targeted to try and optimise HLA matching via banked iPSC lines on a worldwide scale, avoiding redundancy and making the best use of limited resources (Andrews et al., 2014, Turner et al., 2013, Wilmut et al., 2015). Of course, whilst allogeneic cell therapy using HLA haplotype homozygous donors will reduce the risk of direct T cell activation and the influence of preformed anti-HLA antibodies in some patients, it is unlikely that therapies derived from such donors will be completely immune compatible with recipients. However, it is expected that matching will at least mitigate the degree to which immunosuppression is required. Other relevant immune responses include activation of NK cells due to the potential absence of inhibitory receptor ligands on HLA haplotype homozygous donor derived tissues and cells, and recipient T cell
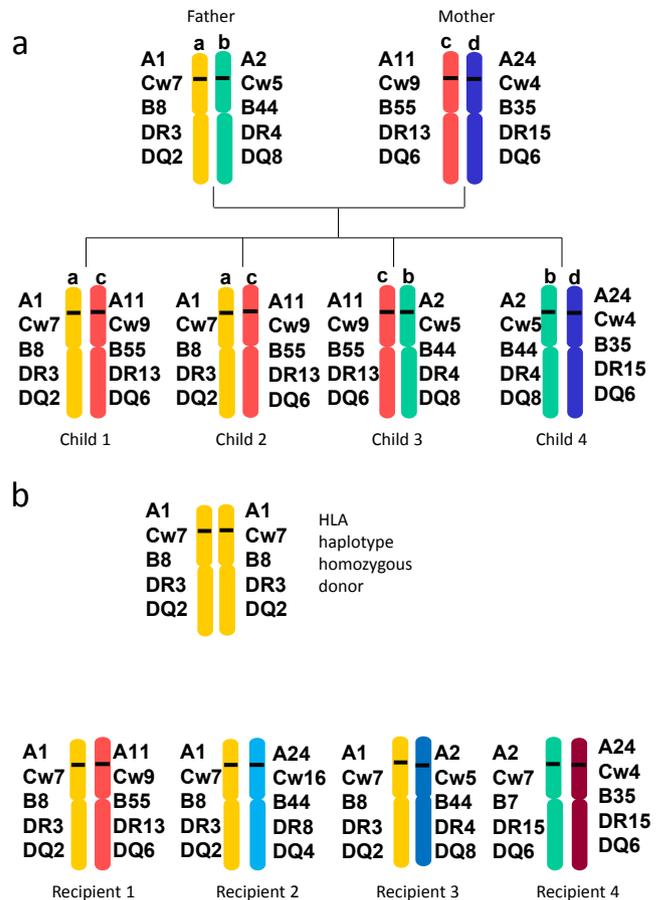

Fig. 1. Alleles at the HLA gene loci are inherited as a block or haplotype. (Fig. 1a). The majority of individuals inherit two different HLA haplotypes and are therefore HLA haplotype heterozygous. In the example given, child 1 and 2 share the same two haplotypes and could provide HLA-matched cells to each other, but not to child 3, with whom they only share one haplotype, or child 4, with whom they share none. However if donors are identified who have inherited two copies of the same HLA haplotype (Fig. 1b) these HLA haplotypes homozygous donors will be able to provide HLA matched cells and iPSC derived products to patients in the population who carry only one copy of this haplotype. In the example provided, this HLA haplotype homozygous donor could provide matched cells to recipients 1, 2 and 3 but not 4. Donors identified who are HLA haplotype homozygous for a high frequency haplotype in a population may match many patients, but as a haplotype becomes less frequent it will i) prove harder to identify suitable donors and ii) they will only match a smaller proportion of patients.

responses against minor histocompatibilty differences between donor and recipient.

3.1. HLA antigen presentation

The classical HLA class I molecules, HLA-A, HLA-B and HLA-C present self and non-self peptides to CD8+ cytotoxic T cells derived from proteins expressed endogenously within the cell. HLA class I molecules are expressed on all nucleated cells, although their level of expression can vary depending on the cell type and the inflammatory milieu. Presentation of non-self peptide will lead to the activation of specific cytotoxic T cells, once cytokine help is received from T helper cells. Also, normal expression of classical and some non-classical HLA class I molecules (HLA-E) presenting self peptides is an indicator of cell health. Perturbations of this expression, caused by viral infection and tumour development, will trigger activation of NK cells.

HLA class II molecules, HLA-DR, HLA-DQ and HLA-DP have a more restricted tissue distribution than HLA class I being expressed on

specialised antigen presenting cells such as dendritic cells, macrophages and B cells. Their expression can be upregulated however on other cell types such as endothelial cells during inflammatory responses. The HLA class II molecules present exogenously derived peptides to CD4+ T cells. This is a critical step in the initiation of an adaptive immune response as the activation of CD4+ helper T cells is required to develop cytotoxic T cell responses and B cell responses.

The role of HLA molecules in normal physiology is therefore to protect cells from pathogens by developing robust immune responses that lead to immunological memory, as well as providing a marker of the health of individual cells. The central role of HLA molecules in the immune response is part of the reason that exposure to foreign HLA during transplantation, transfusion or pregnancy can lead to such a strong anti-donor alloimmune response.

3.2. Alloreactivity

The alloimmune response refers to immunological processes that occur when an individual is exposed to foreign tissue or cells. The response is multifaceted but at least in its early stages the main target of the response is, perhaps unsurprisingly, the human Major Histocompatibility Complex HLA antigens. It is estimated that 1–10% of the T cell compartment can be activated against foreign HLA molecules (Siu et al., 2018) via recipient T cells binding to intact foreign HLA molecules expressed on donor cells. In particular, dendritic cells carried over as passenger leukocytes in the donor organ present intact HLA molecules to naïve T cells upon migration to the secondary lymphoid tissues of the recipient. This 'direct' T cell activation occurs because there are many T cells that have not been rendered tolerant of self peptides capable of binding to non-self HLA molecules, since such peptide-MHC complexes are not present during the process of thymic negative selection. Recipient T cells can also be activated against foreign HLA by a normal immune activation mechanism known as indirect T cell activation. In this situation foreign HLA shed from donor cells is taken into specialized antigen presenting cells and presented to the host immune system via recipient HLA molecules. These two processes, direct and indirect T cell activation, and possibly also a third mechanism named semi direct T cell activation, whereby intact donor HLA is taken onto the surface of recipient dendritic cells via the process of trogocytosis allowing T cell activation (Zeng and Morelli, 2018), provide powerful stimuli to host T cells and maintain early and late immune responses against non-HLA matched tissues and cells (Ali et al., 2013). As well as this important activation of T cells against foreign HLA, the alloimmune response also features activation of T cells against other non-self proteins presented in the context of recipient HLA molecules (collectively known as minor histocompatibility antigens) (Almoguera et al., 2014), NK cell activation (van Bergen et al., 2011), and may involve the presence of pre-formed HLA donor specific antibodies (DSA) or, often later in a response, de novo HLA-DSA (Thomas et al., 2015).

3.3. Mechanisms of graft rejection

The alloimmune response described above may lead to allograft rejection depending on the organ/cells being transplanted. The relative importance of the alloimmune response overall depends on the transplant procedure undertaken. In liver transplantation, for example, there appears to be an ability of the liver to withstand a certain degree of damage caused by T and B cell based immune mechanisms (Del Bello et al., 2016), due to the immune privileged environment it provides and its unparalleled capacity for tissue regeneration, however, even in this case immunosuppressive agents are commonly used to dampen immune responses. In cardiothoracic and renal transplants early direct activation of T cells can lead to acute T cell mediated rejection. The presence or development of HLA-donor specific alloantibodies (HLA-DSAs) is associated with reduced graft survival via early aggressive hyperacute rejection, in the case of preformed high-level HLA or ABO antibodies, or a more gradual insidious chronic damage in the case of de novo HLA antibodies, often directed against HLA class II (Montgomery et al., 2018). In hematopoietic stem cell transplantation

(HSCT), although host immune responses may be of relevance and lead to failed engraftment, a larger issue is the activation of donor immune cells which recognize the recipient as non-self. This may lead to the condition of graft versus host disease (GvHD) which occurs in both an acute and chronic form and is mediated via donor T cell and NK cell responses (Kolb, 2017).

3.4. How can allogeneic immune responses be reduced?

In broad terms there are three approaches that may be used clinically to combat alloimmune responses; i) trying to reduce activation of the immune response by HLA matching between donors and recipients, ii) controlling responses via immunosuppressive drugs, and iii) seeking to induce a state of immunological tolerance. Advances in transplantation throughout the 20th century have been closely linked with the development of new immunosuppressive agents. In solid organ transplantation and HSCT most transplant units use combinations of drugs aimed at targeting T cell activation pathways and immune cell proliferation. A range of monoclonal antibody agents are also available and are often used as induction treatment at the time of higher risk transplantation or to try and reverse acute immune rejection events. While the inherent risks of long-term immune suppression may be warranted for end-stage organ failure, the cost-benefit analysis may be rather more nuanced in the context of cell replacement therapy for non-life threatening diseases such as age-related macular degeneration, or others, such as diabetes, for which effective alternative treatment regimens are readily available. Furthermore, the level of risk to which recipients of cell replacement therapy may be exposed is compounded by the known propensity for tumorigenesis of iPSC: to compromise the capacity for immune surveillance of a patient while implanting tissues with a recognised tendency for transformation would raise significant ethical questions. Given these limitations, alternative approaches to the prevention of rejection, such as the induction of immunological tolerance, are clearly attractive.

Although the induction of tolerance to organ allografts remains the holy grail of transplantation that has yet to be translated to the clinic, cell replacement therapy offers a unique set of circumstances which may be more amenable to immune intervention than conventional transplantation. In particular, tissues differentiated from iPSC will be devoid of endogenous dendritic cells serving as passenger leukocytes capable of stimulating the direct pathway of alloreactivity, responsible for acute rejection of the graft (Fairchild, 2010). Consequently, it is primarily the indirect pathway that is operational in this context. However, tissues derived from pluripotent stem cells have been shown to express significant levels of transforming growth factor (TGF)-β capable of polarising effector T cells towards a regulatory T cell phenotype upon infiltration into the grafted tissue (Robertson et al., 2007; Lui et al., 2010). Consequently, tissues derived from iPSC may actively contribute to their own acceptance by establishing a regulatory T cell repertoire. Given that such a mechanism is unlikely to extend to the acceptance of tissues across a full MHC barrier, the induction of tolerance may best be achieved in conjunction with strategies for HLA matching to reduce as far as possible the immunological disparities between donor and recipient.

In many parts of the world it is considered valid to try and match donor and recipient for their HLA antigens in certain forms of transplantation, such as kidney, in order to reduce immune activation and limit de novo HLA antibody formation, which may both pose problems for the existing graft and also limit future transplant options. Large registry data still shows that matching for HLA antigens has an impact on graft survival in kidney transplantation (Williams et al., 2016b). In HSCT, until quite recently, the dogma has always been that matching for donor and recipient HLA alleles is of critical importance to reduce the risk of GvHD (Pidala et al., 2014). However, the introduction of alternative sources of haematopoietic stem cells, such as cord blood, showed that a degree of HLA mismatch could be tolerated in this setting (Eapen et al., 2014) and more recently the successful use of donors who only share one HLA haplotype with the patient (haploidentical HSCT) has shown that even in this context the importance of HLA matching may be overcome when using specific transplant and immunosuppressive protocols (Ciurea et al., 2015).

Table 1
Summary of higher primate animal models using MHC homozygous donor derived material transplanted into heterozygous recipients.

Paper	Model	MHC matched?	Evidence of immune activation?	Nature of response
Mitzukami et al., PLoS One 2014	Pig iPSC teratoma formation, testes and ovaries	Yes, haplotype homozygous iPSC	Yes	T and B cell responses attenuated, but natural killer (NK) and ecomplement (C') mediated immunity
Kawamura et al. Stem Cell Reports 2016	Macaque iPSC-derived cardiomyocytes into macaque hearts +/- immunosuppression	Yes, haplotype homozygous iPSC	Yes	MHC matched transplantation with single or no immune-suppressive drugs still induced a substantial host immune response to the graft. Indirect T cell activation and NK cell activity
Shiba et al. Nature 2016	Macaque iPSC-derived cardiomyocytes into macaque hearts +/- immunosuppression	Yes, haplotype homozygous iPSC	No	Grafted cardiomyocytes survived for 12 weeks without immune rejection in all five iPSC-CM recipients in this study when given methylprednisolone and itacrolimus
Sugita et al. Stem Cell Reports 2016b	Cynomolgus monkey, iPSC-derived cardiomyocytes and intra-myocardial injection	Yes, haplotype homozygous iPSC	No	No rejection signs in the allografts of the MHC-matched monkeys
Morizana et al. Nature Communications 2017	Cynomolgus monkey, iPSC-derived neural cells, +/- immunosuppression	Yes, haplotype homozygous iPSC	Reduced	One animal showed a slight immune response. Possible mechanisms include an indirect pathway caused by minor antigens or innate immunity caused by NK cells.

3.5. HLA typing

Assays used for genotyping of HLA alleles have developed rapidly over the last few years. The use of next generation sequencing (NGS) kits and platforms for HLA typing are now routine in many transplant units, especially for HSCT where allelic level matching for unrelated donor transplant has traditionally been required (Bravo-Egana and Monos, 2017; Mayor et al., 2015). This means that methods are now widely available that can give definitive, allele level typing for all HLA loci. In the context of solid organ transplantation NGS level HLA typing is not necessarily required although may in the future become routine. As donors and recipients are HLA typed at higher resolution and matched for more HLA loci it would be hoped that more information about the relevance of HLA matching in different transplant contexts will become available and help to limit the use of immunosuppressive drugs by personalising dose and use (Wiebe et al., 2018).

4. Alloimmunity beyond HLA

As already discussed, matching for HLA using haplotype homozygous donors will limit early direct T cell activation and the relevance of preformed HLA antibodies but there will still be a risk of indirect T cell activation via non-HLA minor histocompatibility antigens (mHa) and NK cell activation via lack of self HLA or upregulation of ligands for stimulatory receptors. There is much evidence that mHa are associated with graft versus host reactions in HSCT (Jameson-Lee et al., 2014) although there is less clinical data supporting a role for mHa in solid organ transplantation (van Bergen et al., 2011). However, given the abundance of SNPs that will vary between two unrelated individuals (plus iPSC induced changes/epigenetics), T cell activation via mHA recognition must be expected to occur when transplanting allogeneic tissues derived from iPSC, even when HLA is matched.

The idea of using HLA haplotype homozygous donors also immediately raises the possibility of NK cell reactivity against iPSC derived tissues. This is because of the complex activation of NK cells that involves a balance between inhibitory and activating receptors on the NK cell surface (Long et al., 2013). The ligands for a subset of inhibitory receptors, Killer Immunoglobulin-like Receptors (KIRs), are protein motifs located on certain HLA alleles. When transplanting homozygous donor derived tissue into a heterozygous patient there may be the possibility of NK cell activation in the patient due to the absence of inhibitory receptor ligands on the transplanted tissue.

5. Assessing immunogenicity of iPSC derived cells

At the outset of any potential clinical use of allogeneic iPSC derived cells and tissues the immunological consequences of transplantation should be considered within initial experimentation and during early phase trials. The immunological processes that will be important will vary depending on numerous factors, such as the type of cell that is being transplanted, HLA expression and expression of other relevant co-stimulatory molecules, the transplant location and the level of vascularisation. As allogeneic iPSC derived products have begun to move to clinical use there has been an increase in the interest around immunological factors, although the models available to investigate all aspects of the immune response have been limited. *In vitro* experiments looking at mainly T cell activation give some information about the ability of iPSC derived cells to stimulate immune responses, but lack the subtlety to identify other aspects of the immune response that may be important (Sugita et al., 2016a). Recently a series of higher primate animal models have investigated responses against MHC homozygous tissues (Kawamura et al., 2016, Shiba et al., 2016, Sugita et al., 2016a, Morizane et al., 2017). These have revealed that there is indeed variation in the nature of the immune response dependent upon immunosuppression given and the type and location of the cell transplanted. Table 1 highlights some recent studies and the observed immune responses against transplanted MHC homozygous tissues.

In brief, in the context of iPSC-derived allogeneic therapies, HLA matching is important to limit T cell activation and relevance of pre-formed HLA antibodies. HLA haplobanks containing donors selected mainly based on HLA haplotype could provide a source of iPSC lines to HLA match a proportion of patients. The identification, consent and collection of relevant donor material is likely to require close collaboration with haematopoietic stem cell registries. The use of allogeneic material from HLA haplotype-matched donors will likely reduce alloimmunity but will not eliminate the need for some forms of immunosuppression or obviate efforts to induce immunological tolerance as other aspects of the immune system will still be relevant.

6. Future sourcing of clinical-grade iPSC lines from HLA haplobanks

GAiT (Sullivan et al., 2020) is working with the human pluripotent stem cell registry (hPSCreg) (Mah et al., 2020; Seltmann et al., 2016) to build a searchable database for clinical-grade iPSC lines. The database will contain all the pertinent information for iPSC lines to be included in the GAiT haplobank network that could serve as starting material for allogeneic cell therapy development. Such information includes a standardized name of the cell line (Kurtz et al., 2018), donor selection and screening criteria, permissiveness of donor consent, immunological characteristics (including ABO group and HLA haplotype), iPSC derivation and expansion processes used, critical quality attributes [as described in the published GAiT guidelines (Sullivan et al., 2018)], and regulatory acceptance (Barry et al., 2015).

7. Quality standards and the generation of clinical-grade iPSC lines for deposition in a haplobank

It would be a waste of resources and effort to initiate 'GMP' production of iPSCs from donor cells that fail to meet the international regulatory compliance requirements for starting materials for advanced therapeutic medicinal product manufacture. There are mandatory standards for donor selection, screening and procurement of starting material in most jurisdictions, though these are not necessarily completely aligned. If the intent, therefore, is to generate clinical-grade iPSC for global use, attention must be paid to meeting the requirements of both local and major international regulators. Where parties are considering the development of clinical-grade iPSC donor consent also requires particular attention.

The consent required to donate cells or tissue for research purposes differs from that required for the donation of cells or tissues for direct clinical use or the development of cell therapeutics (Lo and Parham, 2009). Specifically, there is a requirement for traceability between donor(s) and recipient(s) which means that abnormalities in tests for infectious disease markers or genetic abnormalities with potential clinical significance need to be communicated back to the donor. Furthermore, consent needs to have been given for commercial development, nonclinical development and testing and clinical use, potentially on an international scale. It is therefore imperative to make sure that appropriate comprehensive donor consent is in place before resources are spent on derivation, or re-derivation (see below), of clinical-grade iPSC lines. Exemplar donor information and consent forms are available on the GAiT website (www.gait.global) but such documentation will need to be further adapted to fulfil local legal, cultural and other requirements.

The generation of clinical-grade iPSC lines is highly expensive and time consuming and should be undertaken in a facility with experience in this kind of work under the aegis of an established Quality Management System and in compliance with local and international regulatory requirements (Siu et al., 2018).

8. Problems with re-deriving research-grade iPSC lines for deposition in clinical haplobanks

Re-derivation of an iPSC line involves a retrospective evaluation and risk assessment of the provenance of a research-grade iPSC line. It has the advantage of not requiring a large outlay at the beginning of the derivation process. For example, the original human embryonic stems cell (hESC) lines were not derived with the intention of being used for the generation of clinical products, but due to the limited access to IVF embryos and the wide use of lines to develop manufacturing processes, some have been used in this way. The H1 hESC line was subsequently rederived and used in part of Phase II Astellas Pharma's retinal pigment epithelial cell (RPE) trial and the rederived H9 hESC line was used to generate dopamine neurons for BlueRock Therapeutics' clinical trial for Parkinson's disease. However, whilst this approach is possible, it is not recommended due to the difficulty in achieving comprehensive retrospective data collection and the inability to 'tests quality into the product'. In reality the challenges of 're-deriving' an iPSC line to clinical-grade may be just as onerous as prospectively generating a new clinical-grade line.

9. Future steps towards comparability

A major consideration for those seeking to develop iPSC therapeutics is comparability, which will allow individual iPSC lines to be substituted for each other when manufacturing a cellular therapeutic without the whole non-clinical, clinical trial and regulatory processes having to be repeated from the beginning. This requires the collection of evidence showing that different cell lines have similar critical quality attributes and that the same product can therefore be manufactured from different starting materials (Barry et al., 2015, Williams et al., 2016a).

Assessment of comparability requires a clear understanding of the critical quality attributes of the cell line, the analytic processes used to evaluate these and the standards required. Key parameters may include microbiological safety, genetic stability and evidence of pluripotency. The GAiT quality standards and regulatory compliance working group are considering the tests required to assess comparability of different clinical-grade iPSC lines. A summary of these are included in Table 2 and the details have been published (Sullivan et al. 2018). GAiT is also liaising with a range of other banking and standardisation organisations working in the cell therapy space to understand the process by which this has been done for other cell types.

Many of the mandatory quality tests for iPSCs lack pharmacopeial guidance which makes participation in Quality Assessment Rounds prudent to ensure quality control testing is consistent between laboratories.

10. Selection of suitable donors for haplobanks

As described previously the high degree of HLA polymorphism in the human population means that most individuals will inherit two different haplotypes from their parents. There is likely an evolutionary advantage to this as inheriting two alleles at each HLA locus gives an individual the maximum capability of recognising foreign peptides and combating infection. There is evidence for this in studies of HIV infected individuals and this selection for heterozygous advantage may in part account for the high degree of polymorphism in the HLA system (Carrington et al., 1999). The concept of haplobanking requires the identification of individuals who are HLA haplotype homozygous. Unfortunately, because this situation will only occur in very few individuals, even for common HLA haplotypes, large numbers of potential donors need to be screened to identify such cases. However, a resource exists that is of use in identifying HLA haplotype homozygous donors; global HLA typed haematopoietic stem cell registries and cord blood banks. There are over 37 million individuals who are registered with

Table 2
Critical quality attributes for clinical-grade iPSCs.

Attribute	Test	Status	Recommended analytical method	Acceptance criteria
Identity	STR	Mandatory	STR profiles Performed by accredited laboratory	Compare donor material with iPSC line.
Microbiological sterility	Mycoplasma	Mandatory	Qualified qPCR or culture (broth/agar or Vero inoculation/DNA stain) method Use of pharmacopoeial methods USP < 63 >, Ph.Eur.2.6.7 and JP17 < G3 >	Negative
	Bacteriology	Mandatory	Use of pharmacopoeial methods USP < 71 > and < 61 >, Ph.Eur.2.6.27 and 2.6.1, JP17 < 4.05 > and < 4.06 >	Negative
	Viral testing	Mandatory	Based on risk assessment of starting and raw materials Use pharmacopoeial methods USP < 1237 >, Ph.Eur.2.6.16, JP17 < G3 >	Negative
Endotoxin	Endotoxin	Mandatory	Use pharmacopoeial methods USP < 85 >, Ph.Eur.2.6.14, JP17 < 4.01 >	Negative
Genetic fidelity & stability	Residual vector testing	Mandatory	Appropriate specific assay to be used	Negative
	Karyotype	Mandatory	G banding	Normal ≥20 metaphases
	SNP arrays	For information		
	WGS/WES cancer associated panels and other genetic, and disease marker analysis	For information		
Viability	Viability	Mandatory	Dye exclusion test on recovery of a typical iPSC culture after 48 h in culture Use pharmacopoeial methods USP < 1046 >, Ph.Eur.2.7.29	
	Doubling time	Not required Data may be added for information		
	Cell debris	Not required		
Characterisation	Flow cytometry	Mandatory	A minimum of two markers from an accepted panel (SSEA4, TRA1-60, OCT4, Nanog etc.), Use pharmacopoeial methods USP < 1027 >, Ph.Eur.2.7.24	Markers should typically be positive on > 80% of cells in the Master Cell Bank
	Immuno-cytochemistry	For information		
	Differentiated cells	Not required, for information		
Potency	Phenotypic	Mandatory	EB formation and/or directed differentiation. Teratoma formation not required as a potency assay.	Demonstration of cells from all 3 germ layers
	Molecular	For information	Pluritest™ or hPSC Scorecard™	

unrelated donor registries around the world. Although the majority of these donors are HLA typed using molecular PCR based methods, there are only a proportion that have been typed at higher resolution using, for example, NGS technologies, at all relevant loci. However, these HLA typed individuals appear to be the best option for identifying haplotype homozygous donors that could provide starting material for iPSC cell lines to stock a global haplobank.

Local HLA population data, as well as large registry data can be used to identify those HLA haplotypes that are common in different populations. However, if all geographical regions pursue this approach in isolation then there will be duplication of work as groups will develop iPSC from donors who have the same HLA type. It is hoped that a large analysis of haplotypes in different geographical regions will identify overlap in utility and that a truly global list of haplotypes can be created allowing the production of expensive iPSC lines to be targeted by different groups, ultimately creating a virtual bank of lines for use worldwide.

As well as selection based on HLA, other factors should also be taken into account when identifying suitable donors for haplobanking. Selection of blood group O donors will enable the use of tissues derived from the donor in all recipients irrespective of blood group and presence of ABO isoagglutinins. Additionally, where possible, selection of female donors may be preferable to limit the potential reactivity of female patients against minor histocompatibility antigens (mHa) encoded by genes on the Y chromosome (Zimmermann et al., 2012).

11. Accessing Haematopoietic Donor Registry data to identify suitable donors

Many studies have already described HLA haplotype frequencies in different populations and useful haplotypes can be identified that will have local utility and also be useful across populations (Lee et al., 2018). However, in order to identify the optimal haplotypes to select for haplobanking on a global scale it would be informative to undertake a global collaborative haplotype frequency analysis using high resolution typing data for at least HLA-A, B, C, DRB1 and DQB1 across different populations. Analysis at this level may reveal allelic differences between haplotypes that are shared across populations that could have clinical consequences. Collaboration with haematopoietic stem cell registries and bodies such as the World Marrow Donor Association (WMDA) is therefore required. Release of this data for analysis will require consent from registries and efforts to allow this are ongoing between GAiT and registries. This analysis would also be able to confirm the existence of actual haplotype homozygous donors that could be approached in due course to consent for cell donation for iPSC production.

12. Conclusion

Haplobanking iPSCs requires substantial expertise in a number of different areas including induced pluripotent stem cell derivation and characterization, GMP-grade cell manufacturing and immune matching. Many stakeholders underestimate three areas pivotal to iPSC haplobanking for clinical purposes and their consequences: (i) the polymorphism of HLA gene system, (ii) donor consent, selection and screening and (iii) the requirements for manufacturing iPSC lines under GMP conditions. Demonstrating the comparability of lines as a starting material for cell therapy development is a complex process. Stakeholders with a long-term vision and capacity to generate lines under GMP conditions will be able to maximise the immune matching coverage of their patient populations through the sharing of lines provided that the community can agree a common set of critical quality attributes and standards.

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