Health by Advanced Therapies

# D2.2 Induced Pluripotent Stem Cells as Universal Allogeneic Resources for Advanced Therapies

Public

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## 1. Deliverable's description

During the last two decades, several major technological breakthroughs have revolutionized the field of cell therapy. The major event was the discovery of Induced Pluripotent Stem Cells, (iPSC) by the Nobel Prize Laureate, Shinya Yamanaka et al (1) initiating a novel and groundbreaking period of scientific progress. This major achievement has already led to several cell therapies approaches, some of which, are at the stage of "first-in-man" trials. We can currently state that we are at the dawn of iPSC-derived cell therapies. The major clinical promise of these advanced therapies in the field of regenerative medicine and oncology, prompted the creation of European and International consortia that developed attractive cell-derived products to be manufactured from large scale clinically compliant and accessible iPSC banks. Remaining challenges are outlined below for large-scale application of these 'living drugs' in humans, in particularly with regard to the manufacturing capacity of safe pluripotent stem cell lines as robust 'starting material' to lead a viable IPSC-derived cell product into market.

## 2. State of the art

In less than two decades, the breakthrough of reprogramming technology opened novel potential paradigms of cell therapy approaches which could hardly have been imagined in the 20th century. The unlimited self-renewal potential of iPSC and maintenance of pluripotency during iterative passages are quite similar to human embryonic stem cells (ESC), branding them as ideal starting cells to generate all types of differentiated body cells, as well as functional structures such as organoids.

A limited number of pluripotent stem cell therapy trials are currently under way, targeting diseases with high unmet medical needs. Early clinical trials used initially ESC for treating a variety of incurable disorders, such as retinal degeneration, end-stage heart failure, diabetes and Parkinson's disease (2). The first clinical use of iPSC has been reported in 2014 by Takahashi et al. (Riken Center, Japan) for neo-vascular age-related macular degeneration by transplantation of autologous iPSC-derived retinal pigment epithelial (RPE) cell sheets (3). The graft remained intact after one year, without hyper-proliferation or tumorigenicity. Visual acuity did not improve or worsen. However, autologous RPE production being too long and expensive, a new clinical trial was launched with allogeneic RPE derived from HLA loci-matched iPSC line, after successfully transplantation in NH primate model (4).

Since the first demonstration of the feasibility of this iPSC study, new pivotal iPSC-derived therapies are now evolving rapidly. A range of pre-clinical and early-stage phase I/IIa interventional studies are currently in progress, mostly in Japan and USA, exploring the tolerance and benefits of various allogeneic iPSC-derived cell types such as: iPSC-derived RPE (Retinal disorders, Riken Center) iPSC-derived MSC (for Steroid-Resistant GVHD, Cynata Therapeutics), dopaminergic progenitors (for Parkinson's disease, Kyoto University), cardiac progenitors (for

Heart failure, Osaka University), platelets (Kyoto University), beta pancreatic cells (for type I diabetes) and engineered NK cells (for advanced solid tumors, Fate Therapeutics).

Expected immunogenicity of allogeneic differentiated cells requires obviously systemic immune suppression therapy, leading to long-term risks of infection, neoplasia and cardiovascular diseases. Minimizing the extent of immunosuppression will therefore be a key feature for widespread adoption of these allogeneic therapies, from the perspectives of safety, efficacy and worldwide health economy.

## 3. Challenges and limitations

iPSC-derived allogeneic therapies are limited by high production costs, limited starting materials and raw materials compliant with industry standards. Stable cGMP compliant iPSC lines and agreed CQAs are required for current developments of AMTPs. The cost of goods and the process complexity will constitute major challenges for the commercialization and competitiveness of iPSC-derived allogeneic therapies. This will require automated processing solutions and controlled scalable automated process solutions for producing a billion of IPSC and derived cells.
Although pluripotent cell lines are considered to exhibit promising advantages from the manufacturing perspectives, critical challenges and considerations need to be met for placing the future iPSC-derived cell therapies in the market.

### I- How to reduce the immune reaction of allogeneic IPSC-derived therapies?

For a large scale therapeutic landscape, immune-HLA matched IPSC lines suitable for a wide variety of HLA genotypes would be valuable for a high number of patients.

One strategy is to derive and bank iPSC lines from healthy donors harboring the most common HLA genotypes, or from “super-donors” homozygous at HLA-A, B and DR loci, which will be valuable for all patients heterozygous for that haplotype (5). An average number of 100 HLA homozygous lines would cover 78% of European Americans, 63% of Asian Americans and 45% of African Americans (6). 50 HLA homozygous donors could match over 90% of the Japanese population (7), 150 selected HLA homozygous donors’ iPSC could match 93% of the UK population (8). However, the identification of such donors requires to screen a large number of donors.
Another recent strategy, consists in deriving hypo-immunogenic iPSC lines from a unique donor using engineering approaches to reduce the immunogenicity. Innovative approaches were already reported by inactivation of MHC class I and/or II genes, over-expression of HLA-E or HLA-G genes (9), or the overexpression of Immune checkpoint inhibitors such as PDL1, CTLA-4, CD47. More recently, it has been estimated that genome-edited 12 IPSC lines by selective deletion of HLA- A and B antigens but retention of HLA-C antigen, would be sufficient to cover > 90% of the worldwide population for 5 ethnicities (10). Nevertheless, despite the fantastic progress in gene editing strategies, genomic alterations should be carefully verified, and a safety switch will be designed to secure IPSC-based therapies.

## II- How to reduce the tumor risk?

It is now well established that both ESCs and iPSCs accumulate some chromosomal aneuploidies, especially after prolonged periods of cultures and passaging driven by the selection of genes which promote growth advantage (11), or potential tumorigenesis. Variations of gene sequence and copy-numbers as well as mutations affecting known oncogenes has been reported (12,14). An exome sequencing study of 140 independent human ESC lines revealed in 5 analyzed cell lines, the presence of common cancer-relevant TP53 mutations. The TP53 mutant allelic fraction increase with passage number (15), and highlight the risk of employing PSCs for cellular therapies. Some new assays and procedures will need to be developed in order to reduce the risk of teratoma from persistent residual undifferentiated iPSCs or non-differentiated cells in the population of differentiated cells in the final product administered to the patient.
Those real risks of genomic instability and dynamic appearance of oncogenes in iPSC lines and potentially derivatives, encourage a global organization with regional hubs of iPSC bank repository production and qualification.

## III- How to facilitate regulatory clearance?

Any single line of iPSC bank would need to be thoroughly tested for viral contamination, tumorigenicity and genome instability. A global iPSC bank should be more efficient to provide well-qualified batches of stable and safe starting material. Supporting science in automation technology, novel raw materials and innovative processes will improve the quality of iPSC lines and derivatives, by efficient cutting-edge efficient supply chains, from reprogramming to expansion, within existing infrastructures.
The demonstration of cell comparability between various batches is also a crucial challenge to maximize strategic development of cell therapy. Since key markers and assays may not be available at this time, an important issue is the validation of key international quality standards complying with cGMP, and to define Critical Quality Attributes (CQAs) related to safety agreed by the scientific community and regulatory agencies.

## IV- Access to allogeneic cGMP immune-compatible IPSC lines:

After the seminal intellectual property developed by S. Yamanaka, (Academia Japan), others have followed by protecting new reprogramming methods, modified transgenes, transduction technologies, genomic modifications, cell differentiation protocols. Very few commercialized allogeneic clinical-grade iPSC lines are currently available, with non-exclusive license fee and restriction rights to develop and commercialize a product. Fujifilm Cellular Dynamics International (FCDI) holds the licenses rights to Yamanaka's patents. Through its licenses and intellectual property FCDI has produced by episomal plasmids, few HLA "super donor "cell lines with a genetic matching for 19% of the U.S. population. Very few allogeneic clinical grade-IPSC lines are provided by public research organizations such the NIH for explorative early stage human studies, as well as in Japan (CiRA).
To anticipate future demands in effective allogeneic therapies, a European iPSC bank free-of-operation or accessible rights will be developed through a joint effort from EU countries in a sustainable and flexible ecosystem model in order to facilitate ATMPs and cell therapy development. Those therapeutic-grade HLA-IPSC banks will allow to pre –manufacture and deliver off-the-shelf cell therapy products, easily accessible for critical acute or subacute

diseases or new emergent diseases such as the current pandemic SARS-2-induced inflammatory disorders.

## 4. Putative solutions

Worldwide, well characterized iPSC lines as starting material sources needs to be developed within a global organizations to face emerging scientific and industrial needs.

### I-Building a European clinical-grade HLA matched IPSC repository and universal hypo-immunogenic cells:

Despite accessible research-grade IPS lines in European network banks and stem cell infrastructure, there is no clinical-grade "super donor" HLA homozygous IPS line in Europe. HLA typed bone-marrow cell banks, hematopoietic stem cells registries and cord blood unit banks can provide starting material for iPSC generation. The donor-recipient compatibility issue is being closely monitored in European and International normal donor banks (https://www.ebi.ac.uk/ipd/imgt/hla/stats.html). Strong collaborations with stem cell manufacturers and stem cell /donor registries organization are therefore required. Immune HLA matched lines can be stored and be accessible in the global virtual data library called 'Haplobank'. European centers are implementing strategic development plans and regional hubs to generate those seed HLA-matched iPSC banks according to Good Manufacturing Practice (GMP) in clean room facilities with product manufacturing licenses (16). Manufacturing of scalable unique cell standardized products suitable for various types of diseases and multiple clinical indications should reduce the cost of the final products. From a pharmaceutical point of view, it will, in addition, allow performing randomized clinical trials, testing dose-response effects, and designing combination therapies.

### II-Harmonizing manufacturing, quality standards and regulatory for iPSC-Therapies:

Collaborative work will have a valuable impact in accelerating pluripotent stem cell therapies in this immature field. The international worldwide network "Global Alliance for iPSC Therapies" (GAiT) (www.global.gait) which include European countries, gather a multidisciplinary network of recognized experts to build greater understanding and agreement on Critical Quality Attributes (QCAs). International release criteria for the Haplobank system has been recently reported (17). International External Quality Rounds of a same cell lines between laboratories will ensure reproducible mandatory quality testing assays, for which currently there are no pharmacopeial guidances available.

# 5. Challenges for RESTORE

RESTORE will promote a dynamic and strategic collective work to move forward in the nascent field of IPSC-based therapies with the aim to foster phase I/II trials in incurable diseases, in which unmet needs have been identified. New **tailored, flexible and integrated IPSC manufacture** systems will be designed and developed to enable cost-efficient manufacturing and assessment.

To define a strategy for future translational success, it will be important to consider all aspects of IPSC lines as a global source of 'starting materials' including informed consent donor, safe IPSC processing banking and shipping, standardized raw material, worldwide agreed standards and uniformed regulatory approval.

## I- Reducing the variability of IPSC:

As IPSC lines show greater diversity and heterogeneity as compared to ESCs, novel reprogramming methods and accurate selection methodologies of IPSC clones based on relevant criteria, might improve the quality of IPSC lines. Genetic and epigenetic variations can affect the efficiency of differentiation between iPSC clones, or cause an unexpected phenotype of iPSC-derived cells (18,19).
**Identification of extensive biomarkers** and epigenome signatures highly predictive of differentiation potential, such as the expression of miRNA (20), during reprogramming or at pluripotent undifferentiated stage, or at earliest stage of lineage commitment would be useful to control and select "bona-fide" IPSC clones. Advanced automated reprogramming technologies, expansion methods by further development of cGMP bioreactors, raw materials, innovative artificial intelligence-based and other digital solutions will have an important input on cost of goods and extrinsic variability of cell lines.

**Comparability between iPSC lines** derived from various donors by different manufacturers, and batch-to-batch consistency is an important challenging issue to maximize the use of iPSC lines repository. Potential interchangeability of cell line should allow the generation and delivery of a same product from different banks with the same cell line, or from different cell lines. For this purpose, it will be crucial to collect prospectively numerous data and multiple biological parameters ensuring useful attrition tests, upon agreed mandatory CQAs. An ongoing shared database for clinical-grade iPSC lines including HLA-haplotype data is available with the 'GAIT' and the European Pluripotent Stem Cell registry (www.hpscreg.eu).

## II- Innovative strategies for immune compatible and safe IPSC lines:

Alongside of the construction of the European Haplobank, **universal hypo-immunogenic cell lines** using various gene editing strategies to evade immune radar are complementary clinically useful cell lines in some particular contexts. This strategy could indeed be valuable for rare haplotype cells, and in relevant clinical applications, such as, hematopoietic cell transplantation, where HLA mismatches profoundly affect engraftment, or in autoimmune diseases, where autoantigens presentation would cause side effects. Another advantage of using a universal cell line arising from a single genetic background, is the easier extensive pre-clinical testing reducing expenses and time, as compared to characterization of cell products derived from multiple donors and various cell lines. However, genome editing induce a risk of off-target modifications that must be extensively controlled. Those modifications can enhance the complexity and regulatory delay.

Since immunogenicity differs also between differentiated cell types, novel approaches more suitable with the clinical indication will evolve. In order to avoid both acute and chronic immune rejection responses, combining immunosuppressive strategies will need to be devised, such as, expression of adequate immune regulatory checkpoints or induction immune tolerance approaches. Those **tolerance induction strategies** will include the use of regulatory immune cells (TReg, MSC), small molecules targeting innate and adaptive arms of the patient's immune system, or scaffold protecting cell graft from immune recognitions.

**Ultimately, adequate safety of engineered cell products** must be achieved by suicide genes therapy approaches included in editing steps. Several genetic manipulation strategies have already generated a major impetus for translational research to eliminate the chromosomally abnormal cells and dividing cells harboring a potential risk of genetic alteration, upon transplantation (21,22). Inducible caspase-9 suicide genes, TK gene safety cytosine deaminase/5-flurocytosine, or herpes simplex virus/ganciclovir will be adapted to iPSC lines.

Both non-exclusive models will be enriched by variant models and innovative strategies will evolve as a step towards complete immune-matched iPSC lines with fully personalized therapy. Non-HLA minor histocompatibility antigens from Y chromosome's genes, and SNPs profiling should also be taken in account.

**Pre-clinical relevant humanized animal models** testing the immune rejection risk and the design of **novel immunogenicity assays** will be helpful to select the right iPSC line for the right patient. Depending on costs and clinical context, it may be possible in the future to produce autologous iPSC for individual use.

### III- Bridging the gap in lack of consensus on relevant test for tumorigenicity:

While the risk of teratoma formation arising from iPSC-derived therapies is considered to be low, manufacturers will have to establish methods ensuring the absence of residual iPSCs in the final product.

**Sensitive assays allowing detection and elimination residual undifferentiated IPSC** at single cells or small iPSC clumps will need to be developed and included into CQA for release of clinical products.

**Detecting genomic alterations and mosaicism** at population level, or at single cell level will be in addition required using easily accessible high-throughput sensitive genomic methods (RNA-Seq, NGS, Hi-C, or ChIP-seq). Indeed these genomic data needs to be considered in order to evaluate the risk of tumor transformation of differentiated derivatives. However, translational research studies will remain necessary to determine the harmful relevance of those specific genomic and epigenetic alterations, as well as, their impact on cellular behavior and long-term effects. The recent possibility of modeling cancer using iPSC-derived 3D-organoid culture systems reproducing expected hallmarks of cancer or biomarkers (23,24), should be therefore novel tools of interest, and potentially used as potency assays.

Strongest knowledge in this field is required to agree upon the characteristics of safe pluripotent cells, and their acceptable limit of genomic instability. Generation of a common iPSC-related mutation database and an established standard for screening of genetic variations will be required for further genomic stability evaluation.

## 6. Summary

RESTORE has the goal to develop a comprehensive roadmap for implementation of "living drugs" derived from pluripotent stem cells consistent with the European vision and regulatory

bodies. Strategic allogeneic IPSC therapies from universal and scalable iPSC banks based on a 'centric-product' approach, will drive future growth in the cell therapy field. Such allogenic approaches will probably bring down the current cost of iPSC-based cell therapies as compared to the autologous approach. Because the of nascent nature of this field, harmonization of regulatory guidelines and International/ European coordination are needed to establish consensus criteria of acceptable IPSC lines and raw materials for their use in cell therapy manufacturing. Although, autologous and allogeneic therapies may require different approaches, in both cases, training of manufacturing staff in scientific issues is critical to enable better understanding of the impact of changes/unplanned deviations.

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