

D4.1: Regulatory road map from donor samples to iPSC production for manufacture of cell therapy products

A regulatory roadmap from donor samples to human induced pluripotent stem cell (hiPSC) production requires progression from well-documented source materials to cGMP-compliant manufacturing by implementing rigorous quality control (QC), strict adherence to cGMP, defining critical quality attributes (CQAs), maintaining strict donor traceability, and strict adherence to applicable guidelines (e.g. ISO standards, ICH guidelines), and regulatory agency (FDA/EMA) guidance, focusing on traceability of the cell line origins and final product safety, and potency. Key steps include risk assessment, validated donor screening/consent, xeno-free reprogramming methods (preferably non-integrating), master cell bank (MCB) establishment and characterization, and adherence to ICH guidelines for sterility, safety, and genetic stability, leading to IND/BLA submissions. Compliance requires comprehensive documentation, validated processes, and adherence to regulations like 21 CFR Part 312 for IND submissions. Navigating the highly structured regulatory framework includes the following stages:

1. Donor Qualification & Consent (Starting Material): Must adhere to stringent ethical and regulatory guidelines.

- **Donor selection:** Donors must be screened for high-risk behaviors and infectious diseases (e.g. HIV1/2, HBV, HCV, HTLV-I/II, Treponema pallidum, Trypanosoma cruzi, and West Nile Virus) following FDA/EMA guidelines, and , if applicable. Tissue Procurement must permit compliance with cGMP license regulations by meeting regulatory requirements for starting materials to assure traceability and safety.
- **Informed consent:** Must specifically include authorization for reprogramming cells, potentially modifying them, establishing permanent cell lines, commercial use, and extensive genetic testing (e.g. WGS).
- **Traceability:** Strict coding systems (e.g., pseudonymization) should protect donor identity and are also required to provide robust traceability of cells from donor to final product; This also includes traceability back from raw materials and other input reagents and this traceability is used for risk assessment and for equipment qualification. Of course traceability also applies to the whole manufacturing process via documentation of procedures and facility establishment, monitoring and maintenance.

2. Reprogramming & Line Derivation (GMP Compliance): Transition from laboratory methods to GMP-compliant, methods and reagents. are preferable to help assure process reproducibility and minimize tumorigenic risk.

- **GMP-compliant reprogramming and culture conditions:** Utilization of non-integrating, viral-free methods (e.g., episomal, mRNA), and well-defined, xeno-free, chemically defined media will be valuable to minimize genomic disruption and adventitious agents. Of course, the reprogramming process must be documented and validated.
- **Monoclonality & Quality Control:** Single-cell cloning assures that only one cell clone is selected for manufacturing as a control point to reduce the risk of genetic variants, and potentially

aberrant, clones being present in the final product. Rigorous characterization for stem cell markers, functional assays for pluripotency (see “Identity and Purity” below), cell identity (a precise genetic match with the donor, see below), and genomic integrity (karyotyping or a technique demonstrated to provide be at least equivalent level of characterisation).

3. Characterization & Quality Control (QC): Defining meaningful critical quality attributes (CQAs) and developing specific, quantitative assays to measure the ability of the final product to produce the desired therapeutic effect is crucial for clinical approval. Stringent QC using robust (well validated) assays is essential to ensure safety and efficacy. The precise identification of suitable assays (especially potency assays) may take a considerable amount of effort, and improvements may continue right into the clinical trial (Creasey et al 2019). Development of characterisation and safety testing is therefore recommended to start at the research stage of product development. (Simon et al 2024).

- **Identity & Purity:** Confirmation of cell identity of the manufacturing cell line enables the product developer (manufacturer) to confirm a match with the donor origin and absence of cell line switching at later stages of cell line use). Markers of self-renewal (e.g., OCT4, NANOG) and hPSC nature (e.g., TRA antigens, SSEA-3) are important to assure that the typical features of hPSCs are retained. It is also vital from a patient safety perspective that residual undifferentiated cells are excluded from the final product as these have the potential to cause benign teratomas in patients. Furthermore, the selected manufacturing cell line should not exhibit a transformed state that could generate teratocarcinomas or other malignant phenotypes. These considerations are important to mitigate the risk of product cells giving rise to teratomas or other malignancy in patients. Please note that in general the terms “identity, purity and potency are regulatory terms generally reserved for the final product. Whilst it may be helpful to use similar terms for cell lines, the use of these terms for cell lines should be understood and explained.
- **Safety Testing:** Screening for adventitious agents (bacteria/sterility, mycoplasma, viruses, prions), karyotype abnormalities, and tumorigenicity (see “identity and purity” above)). Key objectives of these tests are to assure acceptable levels of risk of causing patient disease (infection and/or malignancy). Immunological issues and release of other bioactive molecules with potential for adverse effects in patients are also important considerations. Whilst “haplobanking” to better match patient HLA markers, as opposed to use of an allogenic and thus donor-unmatched hPSC, offer a strategy to reduce acute rejection. However, many cell glycoprotein types are involved in immunological rejection and these would not be covered by HLA matching alone and this has been addressed in approaches using so-called autologous hPSCs (i.e. hPSCs derived from the patient). Whatever, patient matching strategy is used this does not exclude the formal possibility of changes occurring in the immunologically active phenotype of manufacturing (e.g. de-novo polymeric structures/proteins) during complex hPSC manufacturing processes and the possibility of such changes may need to be born in mind.
- **Potency Assays:** Developing validated assays that demonstrate the functional capacity of the final cell product . It is important to recognized that the use of the term potency in the stem cell field has a very different formal meaning. For cell banks (see below), it is beneficial to demonstrate that a cell line can differentiate into at least the early stages of tri-lineage commitment as this is a key characteristic of pluripotent cells and furthermore, a single cell bank may be used as the starting material for multiple product cell types (see also “Characterisation & QC” above) A review

of approved cell therapy products in the USA has reported widespread challenges in potency assay development and reporting (Simon et al 2024).

- **Raw Material Sourcing:** Documentation and validation of all materials, particularly serum-free media and growth factors, is key to risk assessment and in particular assuring that components are xeno-free where possible. It is important to remember that certain processing materials and processes (e.g. analytical analysis) whilst not forming a component of the final raw material may impact on its quality and safety.

4. Bank Establishment (MCB/WCB):

Development of a Master Cell Bank (MCB) and Working Cell Bank (WCB) is preferably done as part of assuring full GMP compliance for overall product manufacture. Some national authorities do not accept that a cell bank can be GMP as it is not in itself a product but a starting material. EMA Quality Innovation Group is currently discussing key considerations for GMP implementation in the manufacturing of genetically modified iPSCs. This includes discussing at which manufacturing stage 'principles of GMP' or 'GMP' should be applied.

- **Generating a well-characterized MCB:** this will serve as starting material for clinical-grade, off-the-shelf, cell therapy products; A stable hiPSC line must be fully characterized, including identity testing, sterility, viral and mycoplasma testing, karyotype analysis, and appropriate differentiation verification.
- **GMP Compliance:** The MCB must be produced in a certified, validated cleanroom environment under conditions that would meet the requirements of cGMP. Cell bank production may be included under a GMP manufacturing licence.
- **Stability Studies:** Continuous testing of the MCB to confirm that storage does not affect viability or functionality of the MCB and product. Product developers should establish a formal regime to assure stability.
- **Documentation:** Maintain a complete, traceable, and transparent "paper trail" for the entire process and the banking facility establishment, supporting the Drug Master File (DMF) for regulatory submission.

5. Process Development & Scale-Up: A robust, reproducible manufacturing process must be established, including in-process controls. Identification of critical process parameters (CPPs) to ensure reproducibility, focusing on automated systems to reduce operator-dependent variability.

- Transition from research-scale to GMP-compliant, often closed-system, manufacturing. Key issues to be considered are i) Impact on quality and safety of the GMP manufacturing process and final product of changes to raw or starting materials (including cell line), ii) suitability of analytical systems for GMP use and data recording, iii) shift to GMP increases costs; iv) consider value of an overall manufacturing validation matrix that includes everything including banking and manufacturing facilities;
- Validation of expansion and differentiation protocols.

- **Small Scale Setup:** Initially define the Process Parameters (PPs) and Critical Material Attributes (CMAs) in small-scale GMP-compliant systems (e.g., shake flasks, small bioreactors).

It is useful to determine testing variations in key parameters to assess their impact

- **Scale-Up Technology:** Transition to larger-scale platforms such as automated, closed-system stirred-tank bioreactors or automated adherent culture systems (e.g., bioreactor systems) to ensure scalability. It should be taken into account that there might be potential changes of cell culture conditions due to environmental factors i.e. altered reactions /performance in larger scale or different culture technologies (including automation) regarding gas transfer, chemical mass transfer in the culture, or novel surfaces/leachates.

- **Comparability:** any change in the manufacturing process should be assessed for its potential to impact on the quality and safety of the product to assure that that ongoing product has comparability with the original product. This is a special concern for Haplobanking whereby the manufacturer may wish to have a number of cell substrates ready to make an equivalent product for different patients with different HLA haplotypes.

- **Consistency & Robustness:** Validate that the scaled-up, closed-system process maintains product CQAs, specifically ensuring high cell viability and purity during expansion and differentiation.

- **Purification & Harvesting:** Implement and validate robust methods (e.g., chromatography, centrifugal separation, magnetic beads, flow cytometry) to remove impurities and target cell types, in particular to assure removal of undifferentiated iPSCs and process impurities (e.g. unwanted so-called “side populations”).

- **Quality by Design (QbD):** Utilize QbD to define critical process parameters to ensure consistent, large-scale production of high-quality cell therapy product

6. Preclinical Safety & Efficacy: Extensive *in vivo* and *in vitro* studies must demonstrate safety (e.g., no likelihood of cell line transformation and tumor formation) and efficacy of the differentiated product.

- **Tumorigenicity Testing:** Essential *in vivo* or other studies to assess the risk of tumor formation (teratoma) from residual undifferentiated hPSCs in the product. These will require high-sensitivity assays (e.g., qPCR, imaging) as one stem cell could theoretically generate a tumor. Detection of transformed cells which might cause malignancy is also important and this may require more complex *in vivo*/*in vitro* assays to assure product safety. A range of assays have been proposed based on animal models, molecular assays and other functional assays (e.g. Japanese study led by Yoji Sato) (See also above). A critical issue is whether the results can be directly related to patient risk and at this stage there is no definitive assay which could do this.

- **Proof of Concept (POC):** *In vivo* studies in relevant animal models to demonstrate the therapeutic mechanism, dosage, and efficacy.

- **Biodistribution & Persistence:** Studies to track the migration, engraftment, and long-term survival of the transplanted cells.

- **Toxicology Studies:** Comprehensive studies to determine potential toxicity, including immunogenicity and potential for unexpected tissue formation.

References:

Creasey A, Stacey G, Bharti K, Sato Y, Lubiniecki A. (2019) A strategic road map to filing a Biologics License Application for a pluripotent stem cell derived therapeutic product. *Biologicals*. 59: 68-71; doi: 10.1016/j.biologicals.2019.03.007.

Simon CG Jr, Bozenhardt EH, Celluzzi CM, Dobnik D, Grant ML, Lakshmipathy U, Nebel T, Peltier L, Ratcliffe A, Sherley JL, Stacey GN, Taghizadeh RR, Tan EHP, Vessillier S. (2024) Mechanism of action, potency and efficacy: considerations for cell therapies. *J Transl Med*. 22(1):416. doi: 10.1186/s12967-024-05179-7;

H. Bando, M. Di Piazza, G. Gowing, C. Herberts, S. Jackman, G. Leoni, S. Libertini, T. MacLachlan, J. McBlane, L. Mouries, Y. Sato, M. Sharpe, W. Shingleton, B. Surmacz-Cordle, K. Yamamoto, J. van der Laan, (2020) Safety of cell therapy products: in-vitro methods to assess the tumorigenicity of human cell-based therapeutic products, *Cytotherapy*, Volume 22, Issue 5, Page S42, <https://doi.org/10.1016/j.jcyt.2020.03.039>;

APPENDIX - Formal Definitions:

Starting Material: the initial biological substances—specifically cells, tissues, or vector stocks of human or animal origin—from which the active substance or finished product is manufactured;

Raw material: any ingredient, substance, or biological component used during the manufacturing process—such as growth factors, culture media, or reagents—that does not become an integral part of the final drug substance

Identity: a fundamental quality parameter designed to confirm that the specific biological or genetic material present in the product matches its intended composition

Purity: critical quality attribute strictly defined by the absence or controlled limitation of impurities

Potency: a quality parameter for Advanced Therapy Medicinal Products (ATMPs) is defined as the measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product, which is linked to the relevant biological properties.

Comparability (in the context of ATMPs): the scientific exercise of demonstrating that a product has a comparable quality, safety, and efficacy profile following changes to its manufacturing process or a transfer to additional manufacturing sites;

Manufacturing Consistency: defined operationally as the reproducible performance of the manufacturing process to deliver a finished product that continuously conforms to established specifications;

Critical Quality Attributes (CQA): a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality, safety, and efficacy

Critical Process Parameter (CPP): A process parameter whose variability has an impact on a critical quality attribute (CQA) and therefore should be monitored or controlled to ensure the process produces the desired quality.