

HAPLO-iPS

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Working Group 7: Clinical Application

D7.2: Summary of Safety, Clinical and Regulatory Requirements for Cell Therapy with hiPSC-Derivatives

Safety and Quality Requirements, Clinical Translation and EU Regulatory Roadmap

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1 Introduction

1.1 Executive Summary

This report constitutes Deliverable D7.2 of the HAPLO-iPS COST Action (CA21151), produced by Working Group 7 (Clinical Application). It provides a structured summary of safety, clinical and regulatory considerations relevant to the development of human induced pluripotent stem cell (iPSC)-derived advanced therapy medicinal products (ATMPs), with particular attention to products derived from HLA-homozygous iPSC lines ("haplolines") intended for allogeneic use.

The deliverable is intended to support academic developers, manufacturers and other stakeholders involved in the translation of iPSC-derived products towards clinical application. It combines an assessment of quality and safety requirements for the final therapeutic product, clinical-development considerations from first-in-human administration through long-term follow-up, and a practical regulatory roadmap for development in the European Union. The scope begins from the use of a clinically intended iPSC line as starting material and extends through manufacturing, product characterization, clinical trial preparation, patient administration and post-treatment follow-up. Earlier stages related to donor selection, iPSC derivation and the broader regulatory landscape are addressed in other HAPLO-iPS outputs, particularly D4.2 [1].

Safety and quality considerations focus on critical attributes of the final iPSC-derived ATMP, including identity, purity and cellular composition, residual undifferentiated pluripotent cells, potency, viability and therapeutic dose, microbiological safety, genomic stability and tumorigenicity. The clinical component addresses administration and transplantation, dose selection, immunosuppression, patient monitoring, tumor surveillance, immunogenicity and long-term follow-up. The regulatory component provides an EU-focused roadmap covering qualification of the iPSC starting material, preparation of the Investigational Medicinal Product Dossier (IMPD), staged interaction with regulatory authorities and practical tools for clinical-trial readiness.

Across these areas, the deliverable distinguishes formal regulatory expectations, reported clinical-development practice and WG7 expert recommendations. Particular attention is given to HAPLO-iPS-specific questions, including HLA matching and immunogenicity, comparability across haplolines, genomic stability, donor-to-recipient traceability and long-term monitoring.

1.2 Background and Consortium Context

The HAPLO-iPS COST Action (CA21151) aims to establish a collaborative European framework for the generation and clinical translation of human induced pluripotent stem cell lines that are homozygous for frequent human leukocyte antigen (HLA) haplotypes. Such HLA-homozygous lines are intended to increase immunological compatibility across recipient populations and facilitate the development of allogeneic cell-therapy strategies. The Action also promotes knowledge sharing, methodological harmonization and standards relevant to clinical-grade iPSC generation and downstream therapeutic applications.

Working Group 7 focuses specifically on the clinical application of these haplolines. Its objectives include evaluating the landscape of iPSC-derived ATMP development and clinical trials, identifying safety, clinical and regulatory requirements relevant to clinical translation, and contributing to harmonization of translational approaches. WG7 therefore extends the work of other HAPLO-iPS working groups from generation and characterization of the iPSC starting material towards the differentiated therapeutic product and its clinical use.

1.3 Scope, objectives and methodology

The scope of D7.2 extends from the selection and use of a clinically intended iPSC line through manufacture and characterization of the final differentiated ATMP, preparation for clinical investigation, administration and subsequent patient follow-up. It does not attempt to provide a comprehensive review of iPSC derivation or donor-selection requirements, nor does it reproduce the broader international regulatory landscape addressed by WG4. Instead, it concentrates on downstream translational questions most relevant to WG7: final-product safety and quality, clinical implementation and the practical regulatory path towards first-in-human studies.

The objectives are to: (i) summarize the principal safety and quality attributes relevant to iPSC-derived ATMPs and propose a risk-based approach to final-product control; (ii) summarize clinical considerations relevant to first-in-human administration and subsequent patient follow-up; (iii) provide an EU-focused regulatory roadmap from clinically intended iPSC starting material to CTA submission and beyond; (iv) identify issues specific to HLA-homozygous haplotypes and allogeneic use; and (v) formulate WG7 recommendations and highlight unresolved areas requiring further evidence or regulatory discussion.

D7.2 is complementary to the regulatory work performed by HAPLO-iPS Working Group 4. D4.2 [1] provides a broad review of regulatory guidelines, legislation and professional standards governing the quality and safety of hPSC-based medicinal products across multiple jurisdictions and across the product lifecycle. By contrast, D7.2 is intentionally narrower and more translational: it assumes that a clinically intended iPSC line has been selected and focuses on the subsequent pathway towards an iPSC-derived ATMP and its clinical use.

The deliverable was developed through a structured review of regulatory and professional guidance, scientific literature and publicly available information on clinical development of iPSC-derived cell therapies. Sources included recommendations and guidelines from international scientific and professional organizations, relevant regulatory guidance, review articles, preclinical studies supporting clinical development, publications describing clinical iPSC-derived products and publicly available information from clinical-trial programs. Where available, expert input and communications from developers active in the field were also considered.

The evidence is presented so as to distinguish three categories of information: formal regulatory expectations, reported clinical-development practice and WG7 recommendations. This distinction is particularly important where regulatory guidance remains non-prescriptive or clinical precedents are limited, including residual-iPSC assay sensitivity, interpretation of genomic findings, tumorigenicity assessment, potency strategy and comparability across different iPSC haplotypes.

The regulatory roadmap was intentionally restricted to the European Union and European Economic Area in order to provide a focused and practically applicable framework consistent with the European context of HAPLO-iPS. This allows D7.2 to complement the broader international regulatory analysis performed by WG4 while providing more detailed guidance on the pathway from iPSC starting material to clinical trial application.

2 Quality, safety and clinical requirements for iPSC-derived ATMPs

2.1 Quality Control requirements

Quality control (QC) of the final iPSC-derived therapeutic product should demonstrate that the manufacturing process consistently generates a cellular product with predefined characteristics suitable for clinical administration. According to EMA guidance, content, identity and purity are mandatory quality attributes. Sterility and endotoxin testing are mandatory for sterile products, mycoplasma testing is required for investigational cell-based ATMPs, and a potency test should be included unless otherwise justified. Viability and cell

quantity are also minimum elements of characterization of the cellular component, and clinically relevant non-target cell populations should be defined and controlled where they may affect safety or efficacy. Early-phase acceptance criteria may initially be provisional and refined as manufacturing and clinical experience accumulate [2].

For pluripotent-stem-cell-derived products, these general requirements should be complemented by controls addressing risks specific to the iPSC platform. ISSCR guidance recommends qualified or validated release assays covering identity, purity, sterility, activity and potency, together with sufficiently sensitive assessment of target and off-target cell populations. Residual undifferentiated iPSCs are of particular concern because of their intrinsic tumorigenic potential and should therefore form an important component of the product-control strategy [3].

The control strategy should distinguish qualification of the iPSC starting material, manufacturing-process control and release testing of the differentiated therapeutic product. Sullivan et al. (2018) proposed consensus-based quality-control recommendations for clinical-grade iPSC lines, covering identity, microbiological safety, genomic integrity, viability, the undifferentiated state and differentiation potential [4]. Hägg et al. (2026) subsequently investigated interlaboratory reproducibility of selected iPSC quality-control assays in international Quality Assessment Rounds conducted in 2019 and 2023 [5]. Their findings indicated that flow-cytometric assessment of pluripotency-associated markers was sensitive to procedural differences and improved with harmonisation, whereas targeted qPCR screening of recurrent genomic abnormalities showed comparatively consistent results. These publications support harmonised, risk-based qualification of iPSC starting materials; neither establishes universal release specifications for differentiated iPSC-derived ATMPs. The Sullivan consensus recommendations, including proposed core and information-gathering tests, should not be conflated with legally binding EU requirements.

Madrid et al. provide an important bridge between this general regulatory framework and the practical development of iPSC-derived cell therapies. Their review emphasizes dose or cell content, viability, identity, purity, authentication, potency and safety of the final product, while positioning genomic stability, tumorigenicity, adventitious-agent testing and residual reprogramming systems within a broader risk-based control strategy spanning the cell-bank, process and final-product stages [6]. Accordingly, not every important safety attribute necessarily requires testing on every final-product lot. Some controls may be more appropriately performed at the master or working cell-bank stage, during process validation, or on representative clinical batches. In contrast, attributes that can vary directly in the administered product, including identity, purity, relevant off-target populations, residual undifferentiated iPSCs, potency, viability, therapeutic cell content and microbiological safety, are more directly relevant to final-product release [6].

Potency should likewise be distinguished from clinical efficacy. It represents a laboratory measure of biological activity related to the proposed mechanism of action. For complex cellular products, a single assay may not adequately capture biological function and a justified assay matrix or broader potency-assurance strategy may therefore be appropriate [7].

2.1.1 Identity

Identity testing should demonstrate that the final product contains the intended therapeutic cell type and differentiation state. For an iPSC-derived drug product, identity should therefore primarily reflect the therapeutic lineage and its relevant maturation state rather than merely confirming origin from the correct iPSC line.

Published clinical-development programmes illustrate the product-specific nature of identity testing. In the Kyoto Parkinson's disease clinical trial (UMIN000033564), the dopaminergic progenitor product was assessed by flow cytometry for FOXA2 and TUJ1, with >80% FOXA2+/TUJ1+ cells required, while CORIN was used for enrichment and >90% CORIN-

positive cells were achieved after sorting [8]. In the spinal-cord-injury neural progenitor clinical trial (UMIN000035074/jRCTa031190228), SOX1 was assessed by qRT-PCR, flow cytometry and immunocytochemistry, PAX6 by qRT-PCR, and PSA-NCAM and GD2 by flow cytometry [9]. MyoPAXon (NCT06692426) combined molecular assessment of PAX7 and rtTA with flow-cytometric analysis of CD54 and alpha9beta1 integrin, while Eyecyte-RPE used TYRP1, PMEL17 and RPE65 together with additional molecular and immunostaining markers [10,11]. FT819 used a different lineage-specific strategy, with the final CAR-T product characterized by CD45/CD7 expression, CAR positivity and absence of detectable surface TCRalpha/beta [12].

These examples demonstrate that identity is a common critical quality attribute, although the analytical panel must reflect the intended therapeutic phenotype. Flow cytometry permits quantitative, single-cell analysis, but interlaboratory comparability requires harmonisation of sample preparation, reagents, staining, instrument settings, gating and data analysis. Hägg et al. (2026) demonstrated that standardised workflows improve reproducibility of pluripotency-associated marker testing [5]. Complementary molecular methods and immunocytochemistry may support product characterization.

Genetic authentication of the iPSC starting material should be established by STR profiling (e.g., the 8 core plus additional loci in the ANSI/ATCC ASN-0002 standard) or another justified genotyping strategy, with an authenticated donor or reference and predefined interpretation criteria [116,117]. STR testing supports detection of cross-contamination and misidentification, which remains a well-documented source of irreproducible and unsafe material [118,119], and its use has been explicitly recommended for stem cells as well as conventional cell lines [119]. For genome-edited products, STR should be complemented by targeted sequence verification of the edited loci. Authentication may be controlled at the master and working cell bank or intermediate stage with cell identity being embedded within a broader quality dataset (provenance, pluripotency, genomic stability, sterility) [3,120].

Besides STR, HLA or SNP analyses remain important for chain of identity, particularly in autologous workflows and for HLA matching allogeneic strategies. HLA typing, although can help to confirm sample identity, has a different purpose. It establishes immunogenetic characteristics important for selection and matching of HLA-homozygous haplotypes, but may be controlled at the iPSC-bank or intermediate stage rather than serving as the principal final-product identity assay [4,6].

WG7 recommendation. Phenotypic identity and genetic authentication. Identity assessment should distinguish the intended differentiated therapeutic phenotype from genetic authentication of the iPSC starting line. Final-product assays should use lineage-appropriate phenotypic, functional or molecular measures; orthogonal methods should be considered during development, and routine release methods qualified or validated for their intended purpose. The iPSC starting material should be authenticated against a reference profile using STR profiling or another justified method, with predefined interpretation criteria. For HLA-homozygous lines, HLA typing should verify the relevant haplotype; for genome-edited lines, intended edits and unintended genomic changes should be assessed separately according to risk. Testing and documentation must maintain donor-to-product traceability and should support interlaboratory comparability. The manufacturing chain must be controlled and traceable, and any changes related to cell banking must be documented, while routine lot-release assay should remain practical, quantitative and appropriately validated for the final product.

2.1.2 Purity and relevant off-target cell populations

Purity should describe both the proportion of intended therapeutic cells and the presence of clinically relevant off-target or incompletely differentiated populations. The appropriate panel is product-specific and should reflect populations that could reduce efficacy, alter biological activity or create a safety risk. For intentionally heterogeneous therapeutic products, the objective is not necessarily a single homogeneous phenotype but a predefined and controlled cellular composition [2,3,6].

The Kyoto dopaminergic progenitor product illustrates this well: FOXA2/TUJ1-positive cells represented the intended therapeutic population, while POU5F1 and LIN28-POSITIVE an undifferentiated marker and SOX1/PAX6-positive early neural cells represented an unwanted population and were required to be <0.1% [8]. Eyecyte-RPE used TYRP1/PMEL17/RPE65 to define the RPE population and investigated non-RPE lineage markers during product characterization [11]. The CiPSC-islet product (ChiCTR2300072200) is an example in which the therapeutic product is intentionally heterogeneous: final pre-transplant QC showed 94.5% CHGA-positive endocrine cells, including 56.4% C-peptide/NKX6.1-positive cells, 13.4% glucagon-positive cells and 15.9% somatostatin-positive cells [13]. In this case, purity means a predefined functional endocrine composition rather than a completely homogeneous population. For a new product, both desired and clinically relevant undesired populations should therefore be quantitatively specified.

Residual undifferentiated iPSCs represent a distinct safety concern and are discussed in Section 2.2.3. Other off-target populations should be selected according to lineage biology and manufacturing history and quantified using methods suitable for the final product. Development-stage characterization may use broader phenotypic or molecular panels, while routine release should focus on populations that are clinically relevant and sufficiently variable to require lot-level control.

Markers used to characterize undifferentiated iPSC starting material are not necessarily interchangeable with assays detecting residual pluripotent or incompletely differentiated cells in a final therapeutic product. Hägg et al. (2026) reported persistence of SSEA4 during early differentiation [5]. This may limit specificity when SSEA4 is used alone, while its possible utility for detecting some uncommitted populations requires product-specific validation of sensitivity, specificity and expression in target and off-target cells.

WG7 recommendation. Purity specifications should be product-specific and should quantitatively address both the intended therapeutic population and clinically relevant off-target populations. For intentionally heterogeneous products, predefined cellular composition should be established rather than applying a requirement for a single homogeneous phenotype.

2.1.3 Potency

Potency testing should demonstrate biological activity relevant to the proposed mechanism of action and should not be equated with clinical efficacy. A quantitative functional readout is preferred where technically feasible. For complex cellular products, a single assay may be insufficient and a justified potency-assurance matrix combining complementary functional and phenotypic measurements may be appropriate [2,6,7].

The CAR-NK FT819 product was tested for potency by cytotoxicity against NALM6 leukemia cells and showed potent, consistent effector function [12]. iPSC-derived platelets were functionally characterized using PAC-1 binding and P-selectin/CD62P expression after stimulation, together with ADP/collagen-induced aggregation [16]. The CiPSC-islet preparation underwent actual pre-transplant functional QC and showed glucose

responsiveness [13]. Eyecyte-RPE used secretion of pigment epithelium-derived factor (PEDF) as an RPE-related functional measure during CMC development and batch-release characterization [10]. For a neural progenitor product, potency-related assessment included terminal differentiation using HuC/D, betaIII-tubulin and GFAP, and positive neuronal/glia differentiation criteria [8]. By contrast, the public Kyoto dopaminergic progenitor and the MyoPAXon release QA table identify more than a single routine functional potency assay and their biological activity was supported by broader differentiation and preclinical efficacy packages. For the dopaminergic progenitors, functional competence was analyzed as capacity of the cells to mature into dopaminergic neurons. This included stimulus-dependent dopamine release measured by LC-MS/MS after high-KCl stimulation and whole-cell patch-clamp recordings demonstrating spontaneous and evoked action potentials, together with in-vivo efficacy studies in Parkinsonian animal models [8]. Similarly, for MyoPAXon. Biological activity was supported more broadly by demonstrating the myogenic differentiation capacity of the PAX7-induced progenitors, their ability to generate muscle fibers and engraft into skeletal muscle, and their functional benefit in relevant preclinical models [10]. Thus, for both products, the overall potency-assurance strategy relied on a combination of well-controlled manufacturing, lineage-specific identity and purity assays, differentiation capacity and preclinical functional evidence, rather than on one clearly defined rapid functional assay used as the sole final-product release criterium. Broader differentiation and preclinical efficacy studies may support the potency strategy during development, but these should be distinguished from the assay or assay matrix used for routine lot release.

WG7 recommendation. Potency assessment should be linked to the proposed mechanism of action and should preferably include a quantitative functional readout. Where no single assay adequately captures product function, a justified potency-assurance matrix combining complementary functional and phenotypic measurements may be used. Development-stage functional characterization and preclinical efficacy studies may support the potency strategy, but should be clearly distinguished from routine lot-release assays.

2.1.4 Viability

Viability should be assessed under conditions that are representative of the state of the product at administration. For cryopreserved products, post-thaw testing is particularly important because the nominal cell content before cryopreservation may not reflect the viable therapeutic dose delivered to the patient. The analytical method should be suitable for the final product format and appropriately validated [2,6].

The methods used to determine viability have differed between clinical iPSC products. In the Kyoto dopaminergic progenitor product, progenitor spheres were dissociated into single cells and stained with the LIVE/DEAD Fixable Dead Cell Stain Kit, followed by flow-cytometric analysis [8]. For MyoPAXon, viability was measured using the Vi-CELL XR automated cell viability analyzer based on trypan blue exclusion. Importantly, viability was also followed during long-term cryopreserved stability studies for up to 36 months [10]. For CLS001 iPSC-derived corneal endothelial cells (jRCTa031210199), viability was assessed immediately before shipment for clinical administration. Viability was directly integrated into preparation of the therapeutic dose [14]. In the case of the CiPSC-islet product (ChiCTR2300072200), the viability was measured at two different stages using different approaches. Immediately after thawing the cryopreserved CiPSC-islet cells, using trypan-blue with an automated cell counter. The cells were subsequently reaggregated into islet-like clusters and cultured for approximately three days before transplantation. During the final pre-transplant QC of the reaggregated CiPSC-islets, viability was assessed again, this time using a live/dead staining [13]. This approach is particularly appropriate for multicellular

spheroids because it permits visualization of viable and non-viable cells within intact aggregates rather than relying exclusively on a dissociated-cell count. For 3D / tissue engineered products, other ATMPs have used indirect methods such as ATP release [121] can be used as a surrogate or simultaneously prepared small scale replicates of the construct can be used to assess viability [122].

Simple membrane-integrity or dye-exclusion methods may be suitable for many cell suspensions, while complex three-dimensional products, sheets, spheroids or aggregates may require fit-for-format methods or validated surrogate measurements. Additional apoptosis or functional characterization can strengthen development-stage understanding but should not be imposed as a universal lot-release requirement.

WG7 recommendation. Viability should be assessed under conditions that are as representative as possible of the state of the product at administration, particularly after thawing where applicable. The method should be validated for the final product format, and, where indirect or surrogate measurements are used for complex three-dimensional products, their relationship to the viability of the administered product should be demonstrated.

2.1.5 Cell content and viable therapeutic dose

The release specification for cell content should reflect the actual administered form of the product. For relatively homogeneous suspensions this may be expressed as a defined number of viable cells, whereas heterogeneous preparations may additionally require control of the therapeutically relevant population. Structured products such as sheets, spheroids, aggregates or patches may require dose definition by product units or dimensions together with cell content, viability, composition and structural attributes [2,3,6].

The concept of therapeutic dose therefore integrates quantity and quality of the administered material. This quality-control definition should be distinguished from clinical starting-dose selection and dose escalation, which are addressed in Section 2.3.2.

In some clinical trials examples, the preparation was adjusted after viability determination to inoculate the exact determined dose, the preparation was adjusted to 10×10^5 viable cells/200 μL , and 8×10^5 cells in 160 μL were administered [14]. In the spinal-cord-injury neural progenitor Phase I study, a defined dose of 2×10^6 cells was administered after final-product preparation and QC [8]. MyoPAXon is manufactured as a cryopreserved myogenic progenitor product with predefined clinical dose levels and a release viability specification, so interpretation of the administered dose incorporates the measured viability of the final product [9]. For the CiPSC-islet product, the therapeutic material consisted of multicellular aggregates. Final pre-transplant QC therefore combined 98% viability, endocrine-cell composition, glucose responsiveness and a defined spheroid size of approximately 50–250 μm [13]. Similarly, iCEPS is administered as an intact corneal epithelial sheet, so the clinically relevant dose is represented by the transplanted sheet together with its total cell content, viability, purity and structural quality rather than by a simple cells-per-milliliter value [15]. These examples demonstrate that the release specification for cell content should reflect the actual administered form of the product and should provide confidence that the patient receives the intended quantity of viable, therapeutically relevant cells or cellular units.

WG7 recommendation. The release specification for cell content should reflect the actual administered form of the product and should ensure that the patient receives a defined quantity of viable, therapeutically relevant cells or product units. Dose definition should therefore integrate viability and, where appropriate, target-cell content, cellular composition and structural attributes.

2.1.6 Sterility

Sterility testing should demonstrate absence of viable bacterial and fungal contamination using pharmacopoeial methods or appropriately validated alternatives. Conventional culture-based assays may not be completed before administration of short-shelf-life living products; where this occurs, the release strategy should include justified pre-administration microbiological controls and a predefined procedure for managing a subsequently positive result [2,6]. New methods to detect sterility faster than the classical growth in rich medium are being developed such flow cytometry and, with even more sensitivity potential such as qPCR (e.g. SteriSEQ) or Next Generation Sequencing methods. These new methods will provide almost immediate determination of sterility assuring safe application in the patient, particularly useful for non-cryopreserved products.

WG7 recommendation. Sterility testing should rely on pharmacopoeial methods or appropriately validated rapid alternatives. Where the complete sterility result is not available before administration, the release strategy should include justified pre-administration microbiological controls, documented risk assessment and predefined management of subsequently detected contamination.

2.1.7 Mycoplasma

Mycoplasma testing forms a distinct component of microbiological safety because conventional bacterial and fungal sterility testing may not reliably detect mycoplasma. Pharmacopoeial or validated NAT/PCR-based methods may be used, provided that assay performance is demonstrated in the relevant product matrix and is compatible with the intended release timeline [2,6].

WG7 recommendation. Mycoplasma testing should form part of the microbiological control strategy for investigational iPSC-derived ATMPs using a pharmacopoeial or appropriately validated molecular method. The selected assay should be suitable for the product matrix and compatible with the intended release timeline.

2.1.8 Endotoxin

Endotoxin testing controls pyrogenic bacterial lipopolysaccharides that may remain even in the absence of viable microorganisms. A reported method for endotoxin testing used in iPSC-derivatives clinical trials is ALA turbidimetry [8, 10]. Acceptance limits should be justified according to maximum clinical dose, formulation and route of administration rather than transferred directly from another cell product [2,6]. Moreover, matrix validations needs to be performed.

WG7 recommendation. Endotoxin testing should form part of the microbiological release strategy for sterile iPSC-derived ATMPs using a pharmacopoeial or otherwise appropriately validated method. Acceptance limits should be justified on a product-specific basis according to dose, formulation and route of administration.

2.1.9 Adventitious agents

Adventitious-agent risk, including viruses and transmissible spongiform encephalopathy (TSE/prion) agents, should be controlled across the manufacturing chain rather than assumed to be a universal every-lot final-product test. Viral risk is primarily controlled through qualification and testing of the donor material and iPSC cell banks, testing of raw materials and relevant manufacturing intermediates, and targeted or broad viral detection methods according to the identified risk [2,4,6]. TSE/prion risk is primarily assessed at the donor cell procurement stage and for the ATMP development it is primarily preventive and depends on selection and documentation of materials of animal origin [2,4,6]. Where the

raw-material assessment identifies a residual risk, it should lead to defined mitigation measures rather than additional downstream testing alone. These measures may include, replacement of materials of human or animal origin with recombinant or chemically defined alternatives, use of materials that have undergone validated viral inactivation or removal (for example gamma irradiation or nanofiltration), and lot-specific testing or certification in line with Ph. Eur. 5.2.1.

WG7 recommendation. Adventitious-agent testing should be defined through a risk-based control strategy that identifies where contamination could be introduced and at which stage it can be most effectively detected or prevented. Routine final-product testing should not be assumed where the relevant risk is more appropriately controlled at donor, cell-bank, raw-material or process level.

2.1.10 Conclusions on QC requirements

Taken together, the final-product release strategy should focus on attributes that directly characterize the administered product: identity, purity and relevant off-target populations, residual undifferentiated iPSCs, potency, viability, therapeutic cell content or viable dose and microbiological safety, including sterility, mycoplasma and endotoxin as applicable. Complementary genomic, tumorigenicity, adventitious-agent, reprogramming, genome-editing, process-residual, stability and administration-format controls, widely address below at section 2.2 and 2.3, should be positioned at the stage where the corresponding risk can be most effectively characterized and controlled [2,3,6].

WG7 recommendation. Final-product release specifications should be adapted to the therapeutic cell type and focus on attributes that directly characterize the administered product, while complementary safety controls should be distributed across starting-material qualification, process validation, representative-batch characterization and final-product testing according to risk.

Regarding interlaboratory harmonization, HAPLO-iPS should encourage harmonised analytical procedures and periodic interlaboratory quality assessment exercises using appropriate shared reference materials. Initial priorities include authentication, expression of pluripotency-associated markers and screening for recurrent genomic abnormalities in iPSC starting material. For differentiated products, comparisons should be adapted to the therapeutic lineage, manufacture and intended application [4,5].

2.2 Safety requirements: iPSC-ATMP-specific considerations

Beyond the core safety final-product release panel, additional safety assessments should be incorporated according to risks introduced by the iPSC starting material, manufacturing process, duration of culture and expansion, genetic manipulation, product format and route of administration. Their applicability and testing stage should be justified according to product-specific risk rather than assuming that each parameter requires routine every-lot final-product testing.

2.2.1 Tumorigenicity

Tumorigenicity is a central safety consideration for iPSC-derived ATMPs, but it comprises mechanistically distinct risks. Residual undifferentiated pluripotent cells may generate teratomas, whereas abnormal, transformed or clonally advantaged differentiated or partially differentiated cells may produce other forms of unwanted proliferation. The addition of safety switches into the iPSC line to allow elimination of administered cells do not replace residual-iPSC control or tumorigenicity assessment.

Malignant transformation arising from genetic or epigenetic aberrations acquired during reprogramming, extended culture, gene editing, or manufacturing (e.g., recurrent copy-number gains, activation of oncogenes, or residual reprogramming transgenes such as c-MYC) [79,82]. These risks should therefore be assessed through an integrated product-specific strategy rather than a single universal test [3,6,18, 79]. For example, In the Kyoto dopaminergic progenitor programme, besides residual iPSC and WGS/WES, and the final clinical-grade progenitors were transplanted into immunodeficient NOG mice and observed for 52 weeks to assess tumorigenicity, toxicity and biodistribution [8]. The iPSC-cardiomyocyte-patch programme used a cell-growth assay and a 21-day soft-agar colony-formation assay to detect anchorage-independent growth, followed by transplantation into NOG mice. Purified clinical-grade patches showed no tumor formation, whereas non purified controls containing residual undifferentiated cells readily generated tumors [17].

In-vitro assessment should distinguish assays intended to quantify residual pluripotent cells from assays intended to detect abnormal proliferative or anchorage-independent behaviour. Molecular, flow-cytometric and enrichment-culture approaches can provide sensitive residual-iPSC assessment, while soft-agar or related transformation assays primarily address anchorage-independent growth, whereas its sensitivity for very rare residual undifferentiated iPSCs is limited [29,30,35,37,38]. Thus, they are complementary rather than interchangeable. Digital soft-agar approaches, in which samples are distributed across multiple wells and colonies are detected by imaging, have substantially increased the sensitivity of this assay for transformed cellular impurities [74]. A subsequent multisite study further supported the reproducibility and standardization of the digital soft-agar method [75].

The in vivo tumorigenicity assay has been the most commonly the teratoma formation assay. It remains the reference standard, because it is able to evaluate the integrated ability of a cell product to engraft, survive, and form a mass in a living host [80,85,86]. The cells are administered into immunodeficient mice, usually via subcutaneous, intramuscular, intratesticular, or renal subcapsular routes. Of note, the intended clinical site is often more informative than a generic permissive site. Undifferentiated hiPSCs produce teratomas containing all three germ layers, whereas a differentiated product is assessed for residual pluripotent cells, immature proliferative intermediates, and tumour formation [80,85].

Dose and the observation time are critical. Gropp et al. found that subcutaneous implantation of 10^5 hESCs produced teratomas with nearly 100% efficiency and that as few as about 100,000 cells could form tumours with larger group sizes and longer follow-up. The sensitivity of this method sharply decreases when hiPSCs are diluted in a large excess of differentiated cells [85]. In practice, positive control assays are observed for 8–12 weeks, standard teratoma assays 12–16 weeks, and studies of differentiated products or low-level residual cells extend to 4–6 months and occasionally 6–12 months [80,85]. A robust design includes a positive-control hiPSC line, a negative control, multiple dose levels, several animals and sites per group, and a validated claim of detection sensitivity (e.g., "detects 1 residual hiPSC in 10^4 – 10^5 product cells") [80,85]. Notably, a teratoma reflects tumour-forming capacity and is not synonymous with malignancy; the assay therefore complements, but does not replace, genomic and phenotype-based testing [80,87].

Currently, a conventional two-year rodent carcinogenicity bioassay is not a routine requirement for hiPSC-derived cell products. The two-year assay was developed for systemically administered small molecules and defined biologics, where dose, exposure, and metabolism can be standardized (ICH S1A/S1B) [88]. It is poorly suited to living cell products because the administered entity is a heterogeneous, often human, cell population; human cells may not survive or behave normally in immunocompetent rodents; the relevant risk may be a rare residual hiPSC or transformed subclone; and tumor formation depends on cell dose, immune status, implantation site, and the local tissue environment [80,83]. A

two-year study is now generally requested only in unusual cases in which an unresolved long-term oncogenic mechanism persists [80,83,89].

The design of the in-vivo tumorigenicity study should therefore be adapted to the specific product rather than following a fixed observation period. Relevant parameters include the clinical cell dose, differentiation state, implantation site, persistence of the product, animal model and duration of follow-up, together with appropriate positive and negative controls [18]. This explains the considerable variation in observation periods among published iPSC-derived products. A conventional two-year rodent carcinogenicity study is therefore not generally used as a fixed requirement for these products; instead, the duration should be justified according to the expected biological behaviour and tumorigenicity risk of the administered cells [18].

In conclusion, in-vivo studies may provide complementary evidence regarding tumour formation, biodistribution, persistence or ectopic tissue formation. Study design, model, dose and observation period should reflect the product and anticipated clinical risk; no universal observation period is appropriate for all iPSC-derived ATMPs, and conventional lifetime rodent carcinogenicity studies are not a standard requirement for these products. Where in-vivo studies are justified, their purpose should be explicitly linked to the residual uncertainties that cannot be adequately addressed in vitro [18,29,80,84, 85-87,109].

Cancer-related genomic analysis provides an additional layer of tumorigenicity control. Transformation risk is driven by genetic instability, genomic characterization is crucial to tumorigenicity assessment [79,82]. A critical interpretative point is that not all cancer-related changes are equal. Some recurring CNVs are considered undesirable but are not by themselves proof of tumorigenicity while other bona fide transforming events warrants stricter intervention including e.g. cell line rejection [82,90,91]. Genomic panels should therefore be interpreted as a weight-of-evidence body of data reflecting the change type, clonality, functional consequence, and assay sensitivity, rather than as a binary yes/no tumorigenicity test [82,94].

Genomic integrity is discussed further in Section 2.2.2.

WG7 recommendation. Tumorigenicity assessment should use a weight-of-evidence strategy combining residual-iPSC control, evaluation of abnormal proliferative behaviour, genomic characterization and, where justified by product-specific risk, appropriately designed in-vivo studies. No single assay should be considered sufficient to establish the absence of tumorigenic risk.

2.2.2 Genomic integrity

The downside of hiPSCs' ability to differentiate into practically any type of cell is usually the development of genetic aberrations, which may be due to the prolonged culturing and reprogramming or cell editing [123]. Genomic integrity is a critical consideration for clinical-grade iPSC lines and their differentiated derivatives. Abnormalities may pre-exist in donor somatic cells or arise during reprogramming, culture expansion, clonal selection, differentiation or genome editing. Depending on the phase they may arise, these variations may be carried over from the donor cell or provide selective growth advantage or even represent residual plasmid genetic material integrated into the host genome [4]. These abnormalities may range from whole chromosomal deletions/insertions and rearrangements copy-number variants (CNVs), copy-neutral loss of heterozygosity (LOH), single-nucleotide variants (SNVs) and small insertions or deletions (indels), with diverse methodologies required to capture the different mutational burden and sizes. For instance, karyotyping or fluorescent in situ hybridization and array-based comparative genomic hybridization (aCGH) for karyotypic (i.e. chromosomal) and sub-karyotypic (i.e. large CNVs) aberrations,

respectively; SNV-based microarrays for CNVs and loss of heterozygosity (LOH); whole-exome sequencing (WES) to examine predominantly coding regions; whole-genome sequencing (WGS) for broader sequence coverage; and DNA methylation profiling characterizes epigenetic variation rather than coding mutations. [124].

So, according to the specific origin, phase of development and mutational profile, the genetic aberrations that occur during iPSC reprogramming and expansion can be categorized and detected as follows:

1. *Chromosomal*

a. *Numerical and large structural rearrangements (i.e. aneuploidies, >5-10Mb)*

The most common chromosomal aberrations that have been observed include whole or partial gains of chromosomes, including chr1, 12, 17 or 20 and balanced translocations or inversions, [125]. These should be reported by specific genomic region and variant class rather than collectively described as whole-chromosome abnormalities. Gain of chr12p, includes the 12p13 region related to the pluripotency-associated NANOG/GDF3 cluster [126] and gain of chr17q is driven by key regions e.g. 17q25 or 17q12 that include BIRC5 and GRB7 genes encoding significant regulators of survival [127]. Focal amplifications such as 20q11.21 should also be monitored.

Although it does not apply to every variant, various meta-analyses studies have suggested that these abnormalities are recurrent and may arise due to culture adaptation. Such a process provides mutated cells with a significant proliferative and survival advantage against prolonged culturing and suboptimal conditions, allowing them to rapidly outcompete genetically normal (wild-type) cells in the culture vessel. Besides the selection advantage, these recurring events may be found in specific fragile sites, segmental duplications, or histone gene clusters (e.g., the 1q21-q23 region), predisposing them to structural instability during prolonged in vitro cultivation [128]. Undoubtedly, these aberrations may significantly compromise their utility upon downstream differentiation (e.g. delayed exit from pluripotency, blunted differentiation efficiency, increased risk of teratoma).

b. *Sub-karyotypic CNVs (Kbs to few Mbs)*

In addition to the regions that may be repeated due to whole and short or long chromosomal arm gain, smaller sized sub-karyotypic CNVs, in the form of micro-amplifications or micro-deletions, may be also caused during the reprogramming phase and lead to genetic mosaicism of the early passage iPSCs [129,130]. Specifically, Hussein et al (2011) used high-resolution SNP arrays and showed that significantly more CNVs are present in early-passage human iPS cells than intermediate passage human iPS cells, fibroblasts or human ES cells. Moreover, other groups used digital droplet PCR (Table 1) and showed that some of these sub-chromosomal CNVs are associated with parental somatic mosaic events, with, for example, at least 50% being present as low-frequency somatic genomic variants in parental fibroblasts, and are manifested in iPSC lines owing to their clonal origin [131].

Regardless of been inherited from parental somatic cells or generated de novo during the reprogramming process, specific recurrent sub-karyotypic micro-amplifications or -deletions map to specific genomic hotspots and might be selected during expansion and differentiation. Their persistence or enrichment during differentiation is product- and lineage-dependent and should not be assumed to occur universally.

Some of the most prominent examples are:

- i) **20q11.21 amplification:** it ranges between 1-3Mb [130,133,134], being present in both hESCs and iPSCs [124], and including *BCL2L1* (encoding for anti-apoptotic protein BCL-XL), can confer a selective survival advantage. The

- extent, clonality and functional significance should be characterized for the specific line.
- ii) **14q32.33 amplification:** Reported gains may include JAG2 (Jagged Canonical Notch Ligand 2), which binds and activates Notch receptors regulating downstream processes, like cell proliferation, differentiation and survival. Positive correlation has been found between abnormal tissue formation and a copy number of 4 and more [135] using retinal pigment epithelium (RPE), cardiomyocytes (CMs), and neural stem cells (NSCs). However, associations between elevated copy number and abnormal tissue formation should be attributed to the specific experimental models and lineages studied rather than treated as universal clinical exclusion thresholds
 - iii) **17q12 amplification or deletion:** The 17q12 region spans a critical genomic segment (roughly 1.4 megabases) including *HNF1B* and *LHX1*, that is prone to rearrangements and associated with several diseases including diabetes, renal diseases, neurodevelopmental disorders [136]. Like 14q32.33, 17q12 has been characterized as a genomic hotspot for tumorigenicity during iPSC formation, with more than 3 copies being associated with abnormal tissue formation, although this observations require interpretation in the particular differentiated-cell models studied; no universal tumorigenicity threshold should be inferred [135].

2. LOH and copy-neutral uniparental disomy

Copy-neutral loss of heterozygosity (LOH) denotes loss of allelic heterozygosity without a change in total copy number, potentially arising through mitotic recombination or other mechanisms. Acquired uniparental isodisomy is one possible outcome, not a synonym for every instance of copy-neutral LOH. Such changes may escape conventional karyotyping and aCGH but can be investigated using informative SNP genotyping combined with quantitative genotyping PCR or appropriately analysed whole-genome sequencing [134].

Moreover, LOH may result from double-strand-break repair following from CRISPR/Cas9 genome editing in iPSCs that could unmask a recessive or tumour-suppressor allele without any accompanying change in copy number. Undesired on-target effects, including large mono-allelic deletions, insertions, or LOH, have been detected in 18% to 40% of homology-directed repair-mediated CRISPR editing of iPSC clones [137]. Frequencies reported for particular CRISPR/Cas9 homology-directed-repair experiments must be interpreted in relation to editing systems, clone-selection procedures and experimental designs, rather than generalized to all clinical iPSC editing.

3. Point mutations and small indels

SNVs and small indels that are commonly and recurrently seen in iPSCs derivatives may derive from low-frequency or mosaic variants already present in parental somatic cells. (e.g., skin fibroblasts or blood cells), or arise de novo during reprogramming and culture while the rest may be acquired as epigenetic modifications during or after reprogramming [138,139]. Indeed, both cellular reprogramming and prolonged passaging may render cells prone to de novo mutation, either due to transient genomic stress, oxidative damage, and structural configuration shifts, or exposure to environmental and replication stresses [138].

These mutations are usually dominant-negative variants in cancer-associated genes. A thorough high-resolution analysis of numerous iPSC exome and transcriptome data showed that 5% of cell lines had acquired *TP53* mutations, probably providing a survival and proliferation advantage to mutated cells [77]. More recently, a strong positive selection has been detected for recurrent *BCOR* mutations, particularly in blood- compared to skin

fibroblast-derived iPSCs [78]. Specifically, 26.9% of these blood-derived lines were positive in *BCOR* variants, resulting in altered cellular transcriptional profiles and impaired differentiation capacity. Moreover, the higher mutational rate of skin fibroblast-derived iPSCs compared to the blood lines pointed to the source of cells and the respective chosen methodology of nucleotide-resolution characterization as a crucial factor for a safe genomic integrity verification of iPSCs. Finally, the mutations during reprogramming may not occur purely at random but may be associated with specific genomic hotspots, which are usually more highly susceptible to nucleotide changes than other genomic regions. For instance, regions related to histone modifications or altered chromatin accessibility may be more vulnerable to mutations developed during reprogramming or prolonged culturing [140]. Identification of a variant does not alone establish malignant potential.

4. Reprogramming-related vectors

Prompt by the propensity of iPSCs to develop aneuploidies, chromosomal aberrations, and point mutations during the reprogramming phase, researchers found that the use of specific reprogramming vectors could be significantly associated with the increased mutational rate during iPSCs formation [141,142]. Integrating retro- or lentiviral vectors show much higher rates of pathogenic CNVs, structural variations, and genetic mosaicism than non-integrating methods, including Sendai virus, episomal plasmids, or mRNA, which remain genetically closer to the native parental cell [142]. For instance, Okita and colleagues (2011) used episomal plasmid vector and showed that it is characterized by a significantly reduced risk of insertional mutagenesis due to the properties of this circular piece of DNA that replicates independently inside a host cell's nucleus without chromosomal. However, episomal plasmid reprogramming does raise concerns regarding the genetic integrity of the resulting hiPSC lines due to the use of a *TP53* short hairpin RNA (shRNA) cassette [142]. The advancement of regenerative science is accompanied by the development of reliable and efficient non-integrating reprogramming methods, e.g. Sendai virus.

5. Editing-induced variations

CRISPR/Cas9 editing during iPSCs formation often results in on- and off-target genetic aberrations that could be missed by the standard PCR and Sanger sequencing methods. For instance, Kosicki et al. (2018) used long read sequencing and long-range PCR genotyping to detect that CRISPR/Cas9 DNA double-stranded breaks (DSB) cause large kilobase-scale deletions and complex insertions and LOH. Perhaps, Cas9 cuts and error-prone non-homologous end joining (NHEJ) could create large deletions or insertions at the cleavage site. These risks arise from the induction of DNA double-strand breaks (DSBs) and are therefore not specific to Cas9. The same considerations apply to other class 2 nucleases used in cell engineering, such as the Type V nucleases Cas12a (Cpf1) and MAD7, which differ in PAM requirement and cut geometry but still act through DSB intermediates..

CRISPR/Cas9 and similar editing tools DNA DSB could result in large chromosomal translocations, p53 pathway activation, and whole chromosomal loss [143–145]. Interestingly, the Cas9 DSB may cause cell cycle arrest and induce a devastating *TP53*-dependent toxic response in iPSCs [145], killing most of the *TP53* wild-type carrying lines in one study [144], counteracting the successful CRISPR/Cas9 engineering process. Successful gene-edited cells that survive the process tend to be those where the p53 pathway is already broken or inactive, raising important safety concerns for clinical cell therapies. To this end, rigorous monitoring of *TP53* genomic and transcriptomic is always suggested to monitor *TP53* inactivating mutations. *TP53* sequence assessment is distinct from off-target discovery genome-wide methods such as GUIDE-seq [146], CHANGE-seq [147] and DISCOVER-seq [148], which nominate candidate off-target sites for appropriate confirmation (Table 1).

6. Mitochondrial DNA mutations

Besides nuclear DNA mutations, iPSCs derivatives may also harbor hetero- or homo-plasmic mitochondrial DNA (mtDNA) mutations that can significantly impact cellular function during cell differentiation. For instance, when iPSCs differentiate into high energy-demanding cells like cardiomyocytes or neurons, the presence of mtDNA, and especially if unmasked, could lead to decreased respiration, impaired membrane potential and impaired cellular function [149]. A study by Kang et al (2016) showed that the frequency of mtDNA defects in iPSCs increased with age, and many mutations were non-synonymous or resided in RNA coding genes, leading to respiratory defects [150]. Assessment of mtDNA variants and mitochondrial function should be justified according to donor background and product biology.

Table. Complementary analytical approaches for genomic characterization of iPSC starting material and derivatives.

Method	Detects	Resolution & sensitivity*	Blind spots	References
G-banding karyotype (20–50 metaphases)	Aneuploidy, balanced translocations and inversions, large CNVs	~5–10 Mb; mosaicism ≥ ~10–20%	20q11.21, small CNVs, LOH, SNVs	[92,123,132]
FISH	Targeted CNVs and aneuploidy per cell	Single-cell; low-level mosaicism	Targeted regions only	[92,123,132]
qPCR/ddPCR hotspot panels (e.g., 20q11.21, 1q, 12p, 17q, X)	Recurrent gains, including from culture supernatant	ddPCR detects mosaicism down to ~10%	Non-recurrent changes, balanced rearrangements	[92]; [131,132]
SNP array / aCGH	CNVs, LOH/UPD (SNP arrays only)	~50–100 kb	Balanced rearrangements, SNVs; mosaicism < ~20%	[130,133,134]
Optical genome mapping	SVs, balanced rearrangements, CNVs	Get from paper	SNVs	[151]
WES / targeted cancer panels	Coding SNVs and indels (TP53, BCOR, COSMIC tier genes)	VAF ~5% at high depth	Non-coding SNVs, most SVs	[151]
WGS (short and/or long read, compared with donor germline)	SNVs, indels, CNVs, SVs, LOH, vector integration	Genome-wide; depth-dependent (30–100×)	Low-level mosaics unless sequenced deeply	[151]
Deep amplicon sequencing / ddPCR	Low-frequency TP53 hotspots, on-target outcomes	~0.1–1% VAF	Targeted only	[78,132]

Off-target discovery (GUIDE-seq, CHANGE-seq, DISCOVER-seq) plus amplicon verification	Editing off-targets	Genome-wide nomination	Needs orthogonal confirmation	[146–148]
Long-range PCR / long-read sequencing / UDiTaS / CAST-seq	Large on-target deletions, translocations	kb to Mb scale	Must be designed per locus	[143,152,153]
Vector-specific qPCR, integration site analysis (LAM-PCR, TLA)	Residual integrated reprogramming vectors or editing plasmids	Copy number per cell	n/a	[37,141,142,154]
Single-cell DNA sequencing / scCNV	Clonal mosaicism, subclones	Single-cell	Cost, amplification noise	[144,145]
mtDNA sequencing	Heteroplasmic mtDNA variants	n/a	n/a	[150]

* Detection limits are assay- and validation-dependent

Based on the aforementioned analysis and the different methods presented, the need to use different complementary methods rather than a single assay to check and verify the genomic integrity of iPSCs and their derivatives is eminent. A stage-appropriate genomic strategy may combine conventional or molecular karyotyping with SNP or copy-number analysis, targeted sequencing and, where justified, whole-exome or whole-genome sequencing. Comprehensive characterization is particularly relevant at the iPSC-bank stage, while additional testing after differentiation should reflect manufacturing history, process-associated risk and the potential for clonal selection.

The strategy should also account for the clonality of the variant. Mutations present in the founder cell of a clone, including intended edits and editing-associated off-target changes, are clonal and are reliably detected by whole-genome sequencing (WGS) at standard depth (for example 30 to 50x). Mutations acquired stochastically during subsequent expansion are subclonal and may fall below the detection limit of WGS. WGS should therefore be complemented by higher-depth targeted sequencing of cancer-associated or driver genes such as TP53 and BCOR (for example approximately 1000x), or by digital PCR for defined loci, performed at the latest appropriate manufacturing stage, to detect low-frequency variants that WGS would miss and that could expand under selective pressure. The targeted panel should be focused on genes with selective-growth or oncogenic potential, rather than open-ended, since most culture-acquired variants are passengers.

There is no universal disqualifying threshold applicable to all genomic findings. Interpretation should consider the affected gene or region, variant type, clonality or variant allele frequency, known biological consequence and evidence that the alteration confers a selective growth or survival advantage. Comprehensive starting-material characterization should be complemented by stage-appropriate additional testing during manufacturing, while WES, WGS or cancer-related panels may provide valuable information, but they should not automatically be treated as universal final-product release tests.

WG7 recommendation. Genomic integrity should be evaluated using a risk-based, stage-appropriate strategy combining complementary cytogenetic and molecular methods. For HLA-homozygous iPSC banking, harmonised test protocols and comparable reporting formats should be encouraged. Targeted screening of recurrent abnormalities can supplement, but not replace, broader characterization. Decisions about the acceptability of individual variants should reflect biological and clinical relevance rather than the mere presence of variation.

2.2.3 Residual iPSC

Residual undifferentiated iPSCs represent a specific teratoma risk and should be controlled through both manufacturing-process mitigation and sensitive analytical verification. To align with current expectations from the European Medicines Agency (EMA), the U.S. Food and Drug Administration (FDA), international scientific societies, and clinical trial precedents, compliance should not rely on a single analytical method. Manufacturers are expected to demonstrate effective in-process depletion of residual pluripotent stem cells and perform highly sensitive lot-release testing using a combination of phenotypic, molecular, and functional assays.

One approach to eliminate the risk of residual iPSC is in-process depletion. Differentiation and culture conditions, physical or antibody-based separation, selective cytotoxic or metabolic approaches and genetic safety switches have all been explored [19-28]. Any additional depletion step should itself be evaluated for process complexity, residual reagents or new safety risks and should not be imposed universally.

The most common approach is to the assessment of residual iPSC using a method that can provide enough sensitivity for each product and dose. Analytical approaches include qPCR or RT-qPCR, digital PCR/ddPCR, flow cytometry and functional enrichment-culture assays [18,29,30,35,37,38].

To date, no mandatory assay has been established by either the EMA or the FDA; nevertheless, the detection and control of residual undifferentiated PSCs are expected by both agencies. Furthermore, the International Society for Stem Cell Research (ISSCR) and the Health and Environmental Sciences Institute (HESI) also strongly recommend testing for these cells using both in vivo and in vitro methods [18,31]. Notably, the ISSCR recommends mandatory in vivo testing in mouse models [31] and HESI suggests that the most robust in vitro assays for detecting residual PSCs are those based on dPCR and the Highly Efficient Culture (HEC) assay with MACS enrichment, both of which more efficient than in vivo assays [31]. Moreover, HESI recommends that the sensitivity of the selected assay should be validated using spiking experiments for each developed product [31].

Marker performance is product- and matrix-specific and should be demonstrated by appropriate spiking or validation studies in the relevant differentiated-cell background. Reported limits of detection from different studies should not be directly ranked because they often use different biomarkers, matrices, cell backgrounds and assay conditions [18,29,30,37,38].

The required analytical sensitivity should be justified in relation to the total administered cell dose, product format, implantation site and broader tumorigenicity risk [18,29,30]. In-vivo tumorigenicity studies assess biological tumour-forming potential and are not interchangeable with analytical quantification of rare residual pluripotent cells [18,29,30,80,85–8]. Routine lot release may therefore rely on a validated molecular assay where justified, while an orthogonal functional assay can provide complementary characterization when warranted by product-specific risk [18,29,30].

Broader pluripotency assessment frameworks, including molecular classifiers and earlier consensus guidance, have also contributed to the development of approaches for characterizing pluripotent-cell identity and residual pluripotency. These approaches may support development-stage characterization but should not automatically be interpreted as routine residual-iPSC release assays [31,93,103].

WG7 recommendation. Residual-iPSC control should combine effective manufacturing-process mitigation with sensitive analytical verification. Marker selection, assay sensitivity and specificity should be demonstrated in the relevant differentiated-cell background. Where warranted by product-specific risk, an orthogonal functional assay capable of detecting viable proliferative PSCs should complement molecular testing during product characterization. The required sensitivity should be justified in relation to the administered dose, product format, implantation site and tumorigenicity risk.

Table 1. Analytical and functional approaches for detection of residual undifferentiated PSCs and assessment of associated tumorigenicity risk.

Category	Method	Principal readout / rationale	Reported sensitivity / LOD	Strengths	Limitations / role
<i>Molecular</i>	qPCR / RT-qPCR	Detection of PSC-associated transcripts such as LIN28A, ESRRG or other validated pluripotency-associated markers	Commonly reported in the range of approximately 0.001–0.1% , depending on marker and background cell type	High analytical sensitivity; accessible; adaptable to different product types	Marker- and background-dependent; indirect detection; requires validation in the relevant differentiated-cell matrix
<i>Molecular</i>	dPCR / ddPCR	Absolute quantification of PSC-associated transcripts, miRNAs or other validated molecular markers	Reported values down to approximately 0.0001% in optimized systems	Very high analytical sensitivity; absolute quantification; strong analytical backbone	Requires product-specific marker selection, matrix validation and appropriate analytical controls
<i>Molecular</i>	RT-LAMP	Isothermal amplification of PSC-associated transcripts	Reported sensitivity down to approximately 0.00002% in optimized experimental systems	Rapid amplification; potentially very high sensitivity	Less established in routine clinical manufacturing; marker- and matrix-dependent
<i>Phenotypic</i>	Flow cytometry	Single-cell detection of pluripotency-associated surface or intracellular markers such as TRA-1-60,	Approximately 0.1% in representative published systems	Rapid; quantitative; familiar for cell-therapy QC; allows direct enumeration of marker-positive cells	Generally less sensitive than optimized molecular assays; dependent on marker specificity, gating strategy and

		SSEA-4, OCT4 or SOX2			background expression
<i>Functional in vitro</i>	HEC / enrichment-based culture	Selective amplification and colony detection of viable residual PSCs	Approximately 0.0005–0.001% in reported systems; values down to approximately 0.00002% reported with enrichment	Detects viable proliferating PSCs; provides functional evidence complementary to molecular assays	Requires culture time; product-specific validation; not necessarily suitable for rapid routine release
<i>Functional / tumorigenicity</i>	Soft-agar colony formation / digital soft-agar / proliferation assays	Detection of abnormal or anchorage-independent growth	Sensitivity is assay-specific; digital approaches improve detection of rare transformed-cell contaminants, but are not established as residual-PSC LOD assays	Useful for assessment of transformed-cell or abnormal-proliferation risk beyond pluripotency-marker detection	Not specific for residual PSCs; should not be interpreted as a substitute for residual-iPSC quantification
<i>In vivo</i>	Teratoma / transplantation models	Assessment of biological tumor-forming potential in permissive hosts or clinically relevant transplantation settings	Optimized studies have reported detection in the approximate 0.0002–0.001% range, but effective sensitivity is highly model-, dose- and site-dependent	Provides contextual biological assessment of tumorigenicity, persistence and ectopic growth potential	Slow, animal-intensive and variable; not suitable for routine lot release; does not directly quantify rare residual PSC burden

Table 2. Selected clinical-development precedents for mitigation and assessment of residual undifferentiated PSCs.

Programme/ product	Indication/ product type	In-process mitigation / purification strategy	Residual-PSC assessment strategy	Reported specification / finding
<i>Osaka University / Heartseed (Japan)</i> Cardiomyocyte Sheets/Spheroids	Severe Heart Failure	Metabolic Selection: Substitution of glucose for an Acid Lactic-rich medium. iPSCs die due to energy stress as they rely strictly on glycolysis.	Flow Cytometry & ddPCR targeting the cardiac-specific pluripotent cell surface marker CD30.	< 0.1% total CD30+ cells detected in the final lot.[23,25]
<i>Fate Therapeutics (USA)</i> FT516/ FT500 (iNK & iT cells)	Oncology/ Immunotherapy	Extended Differentiation: Multi-week bioreactor progression sequence (iPSC → CD34+ → CD56+), naturally out-competing the undifferentiated population.	Digital Droplet PCR (ddPCR) calibrated to amplify absolute copy transcripts of ESRG and LIN28A.	0 residual iPSCs detected out of 2×10^6 mature immune cells (Sensitivity: 0.00005%).[34]
<i>RIKEN/ M. Takahashi (Japan)</i> Retinal Pigment Epithelium (iPSC-RPE)	Age-Related Macular Degeneration (AMD)	Negative Sorting (MACS): Closed CliniMACS system utilizing microbeams conjugated to TRA-1-60 to physically retain undifferentiated fractions.	Functional Amplification Culture Assay: Re-plating a clinical fraction into mTeSR/Vitronectin media for 14 days to force latencies to proliferate.	Exactly 0 pluripotent colonies clonogenically visualized at Day 14.[35–37]
<i>Mayo Clinic/ NEI (USA)</i> Investigator-led (2024-2026)	Retinal Dystrophies	Enrichment Purification: Optimized monolayer culture and selective enzymatic detachment to eliminate non-differentiated cellular nests.	Dual-Marker qRT-PCR: Simultaneous assessment of ZSCAN10 and LIN28A to eliminate background noise or false positives from pseudogenes.	Limit of Detection (LoD) < 0.01% (Less than 1 iPSC in 10,000 target cells).[38]
<i>BlueRock Therapeutics/ Bayer</i> Bemdaneprocet (BRT-DA01)	Parkinson's Disease	Extended Maturation: Prolonged culture (>30 days) paired with small-molecule inhibitors to permanently arrest dividing pluripotent variants.	Multiplex High-Sensitivity qRT-PCR: Direct quantification of shared transcriptional expression levels of OCT4 and NANOG in bulk cell lysates.	Validated LoD < 0.005% (Fewer than 5 residual iPSCs in 100,000 neural cells).[39,40]

<i>Lund University (Sweden)</i> STEM-PD Trial	Advanced Parkinson's Disease	Inertial Microfluidics: Physical sizing technologies within closed loops to isolate and discard large pluripotent stem cell clusters.	Quantitative qPCR: Real-time screening targeting primary pluripotency transcriptomes (LIN28A and SOX2) correlated with digital flow cytometry.	Compliance verified at < 0.01% presence of active pluripotent transcripts.[41]
<i>Novo Nordisk (Denmark)</i> hPSC-derived Pancreatic Islets (2026)	Type 1 Diabetes	Lineage Constraint Engineering: Genetic modifications to block unintended lineage specifications (e.g., mesodermal traits) during definitive endoderm phases.	Mesenchymal Selective Expansion In Vitro: Forced culture assays designed to robustly expand underlying off-target contaminants.	0 contaminating residual cells visible or detectable post selective amplification windows.[42]

2.2.4 Reprogramming and genome-editing components

Reprogramming-related risk depends on the platform used to generate the iPSC line, including the potential for persistence, genomic integration or reactivation of reprogramming components. For non-integrating methods, sensitive clearance testing is generally most relevant during qualification of the iPSC bank. Additional testing at differentiated-product level should be considered where the reprogramming strategy carries a higher persistence or integration risk or where clinically relevant carryover remains plausible [4,6].

Genome-editing controls should likewise be platform- and process-specific. Potential residual components include editor protein, mRNA or guide RNA, donor DNA, plasmid or viral-vector components and other editing auxiliaries [6,98,105]. Clearance of residual editing reagents should be distinguished from characterization of the edited genome itself. The latter may include confirmation of the intended modification together with assessment of off-target changes, structural variants, copy-number alterations, clonality and broader genomic stability as appropriate [98, 105]. For transient reagents shown to clear rapidly, such as RNP-delivered nuclease and guide RNA that are no longer detectable within days of editing, clearance may be established through process validation rather than routine testing of each lot, particularly where clonal selection and extended culture follow editing. Dedicated clearance testing should focus on components with demonstrated or plausible persistence or integration risk, such as plasmid DNA or viral-vector sequences

Regulatory guidance from both European and US authorities emphasizes characterization of the intended modification and relevant unintended genomic changes [98,105].

Use of a transient editing platform should not by itself be interpreted as proof of clearance. Testing should be positioned at the stage most relevant to potential patient exposure, while repetitive final-product testing may be reduced where robust process validation demonstrates adequate clearance [6,98,105]. Table 3 lists representative option for assessments under a risk-based strategy, not mandatory tests for every product. Testing may be reduced or omitted where clearance is adequately demonstrated by process validation or process knowledge.

WG7 recommendation. Reprogramming- and genome-editing-related controls should be tailored to the platform and manufacturing process. Clearance of transient reagents should be demonstrated at the stage most relevant to residual risk, while genomic characterization should independently confirm the intended modification and evaluate unintended alterations. Routine final-product testing should be applied where clinically relevant carryover remains plausible rather than assumed for all products.

Table 3. Recommended risk-based controls for reprogramming and genome-editing components in iPSC-derived ATMPs.

Component/risk	Representative assessment	Regulatory/control consideration
<i>Cas nuclease or other editor protein</i>	Sensitive immunoassay or targeted mass spectrometry; functional nuclease assay when feasible.	Below a predefined limit justified by clearance kinetics and toxicological risk; absence of residual activity is more informative than concentration alone. Testing may be reduced or omitted where rapid clearance is demonstrated by process validation, as expected for transient RNP delivery.

<i>Editor mRNA and guide RNA</i>	RT qPCR or digital PCR with appropriate extraction controls.	Below the validated limit of detection or a justified acceptance threshold at the relevant release stage, or justified as not required where rapid clearance is demonstrated by process validation, as expected for transient RNP delivery.
<i>Plasmid or donor DNA</i>	qPCR or digital PCR targeting backbone, donor and selectable marker sequences.	No unintended persistence or integration; investigate positive signals with orthogonal and integration focused methods.
<i>Viral vector components</i>	Vector specific nucleic acid assays and replication competent virus testing where applicable.	Meets platform specific regulatory expectations and predefined lot release criteria.
<i>Selectable markers or editing auxiliaries</i>	Targeted nucleic acid, protein or phenotypic assays.	Demonstrated removal unless retention is explicitly part of the product design and risk assessment.

2.2.5 Conclusion on Safety requirements and considerations

Safety assessment of iPSC-derived ATMPs requires an integrated, product-specific strategy because no single analytical or preclinical test can establish the absence of all relevant risks. The principal iPSC-specific concerns include residual undifferentiated cells, abnormal or transformed cellular populations, genomic instability, persistence of reprogramming or genome-editing components and, where relevant, risks introduced by genetic modification or prolonged cell expansion [3,6,18,29,30,84,94].

These risks should be controlled through a combination of preventive manufacturing measures, stage-appropriate analytical testing and preclinical characterization. Sensitive residual-iPSC assays, assessment of abnormal proliferative behavior, genomic characterization and, where justified by the product-specific risk, in-vivo tumorigenicity studies provide complementary rather than interchangeable evidence [18,29,30,74,75,76,82,84,94,109]. The extent and timing of each assessment should reflect the manufacturing history, differentiation state, clinical dose, route and site of administration, expected persistence and biological characteristics of the final product [3,6,18,29,84,101,109].

Safety controls should also be distributed across the development pathway. Some attributes are most appropriately addressed during qualification of the parental iPSC line or master cell bank, others during process validation or representative-batch characterization, and others at final-product release. Repetition of the same test at multiple stages should therefore be based on residual risk rather than applied automatically [2,4–6,44,53].

WG7 recommendation. Safety evaluation of iPSC-derived ATMPs should follow a weight-of-evidence framework in which manufacturing controls, residual-iPSC assessment, genomic integrity, abnormal proliferation, reprogramming or genome-editing-related risks and, where appropriate, in-vivo testing are integrated into a coherent product-specific safety strategy. Unresolved risks with potential for delayed clinical manifestation should subsequently inform the design of clinical monitoring and long-term follow-up.

2.3 Clinical requirements and considerations

2.3.1 Transplantation protocols and LTFU

Transplantation and administration protocols for iPSC-derived ATMPs should integrate product-specific, procedure-specific and treatment-related risks. Potential delayed concerns include abnormal tissue formation or graft overgrowth, tumorigenicity, delayed immune responses where no haplo-identical cells are used, loss or excessive persistence of the graft, risks associated with genetic modification, complications related to the route or procedure of administration and late opportunistic infections [3,69,101].

Where persistence or other delayed risks justify extended surveillance, long-term follow-up (LTFU) should be planned prospectively rather than added only after clinical development has begun [69,100,101,107]. The clinical protocol should define the product and traceability framework, target population, dose and administration procedure, concomitant treatment including immunosuppression where applicable, endpoints, safety monitoring, stopping criteria and arrangements for continued follow-up. Adverse events should be assessed in a manner that distinguishes product-, procedure- and concomitant-treatment-related effects where feasible.

The clinical trial/transplantation protocol must contain following sections:

1. Title and identifiers with specified phase, indication, product code, IND (FDA)/CTIS (EMA) number, LTFU protocol cross-reference number, master and working cell bank identifiers, and donor (HLA) identifiers.
2. Product definition with hiPSC line provenance, reprogramming method, differentiation protocol and stage markers, purification method, final formulation, release specifications including residual-undifferentiated-cell limit, and the traceability code structure.
3. Regulatory classification with ATMP sub-classification with a clear distinction between gene therapy vs. cell therapy vs. genome-editing designation. It must be explicitly stated whether the product is subject to gene-therapy, the LTFU expectations and the reason for these expectations.
4. Target population with inclusion/exclusion criteria, immune-status screening (e.g. haplotyping), and assessed ability of the subjects to comply with 5–15 years of follow-up as an explicit inclusion criterion.
5. Dose and administration with cell dose and potentially dose-escalation rationale, viability and potency of the cells at release, delivery device and route of administration with a potential anatomical target and imaging guidance.
6. Immunosuppression process where needed describing the regimen, start, duration, monitoring of the immune status, prophylaxis, criteria for withdrawal from the study, and post-withdrawal graft-survival assessment.
7. Perioperative monitoring related to risks associated with the administration procedure, ideally imaging confirmation of the delivery, immediate and several weeks safety, and surgical complications attributed separately from the product effects.
8. Endpoints stating the primary safety (phases I and IIa) and efficacy outcomes. Also, exploratory graft-survival and immunology endpoints are to be included along with pre-specified handover endpoints into the LTFU protocol.
9. Safety governance that should include the “stopping” rules, dose-limiting toxicity definitions, data monitoring charter, predefined adjudication of product-attributable vs. immunosuppression-attributable (where relevant) vs. procedure-attributable events.

10. Consent and LTFU linkage with LTFU explanation, patient re-contact permission, sample banking, autopsy provision, patient card issuance at discharge; explicit statement that LTFU enrolment is part of trial participation [100].
11. Traceability that must ensure the donor-to-recipient and recipient-to-donor reconstruction procedure, record retention period, SEC/SoHO compliance, and recall procedure [46,114,115].
12. Data and statistics for population's analysis, handling of subjects who discontinue the interventional phase while they still remain in LTFU, and interim and final safety and efficacy analyses.
13. Discontinuity plan where a custodian for long-term data should be nominated, the procedure for transfer or discontinuation of the study is to be included with a commitment to consult FDA or EMA [100].

The duration and intensity of LTFU should be product-specific. Relevant determinants include expected engraftment and persistence, proliferative and tumorigenic potential, genomic or genome-editing risks, immunological considerations, reversibility of adverse effects and feasibility of monitoring. Gene-therapy-specific follow-up periods should not be extrapolated automatically to all iPSC-derived products; they should be applied when the product falls within the relevant framework or where a comparable delayed-risk profile provides a scientific justification [69,100,107].

Route-specific surveillance may include imaging, functional assessment, graft monitoring and procedure-related safety measures. Consent, traceability, data governance, patient re-contact and responsibility for long-term records should be established before treatment when extended follow-up is anticipated [3,69,101].

Table 4. Design and surveillance considerations for selected transplantation routes of iPSC-derived ATMPs.

Route / site	Typical product	Delivery and confirmation	Delayed-risk focus and surveillance modality
<i>Intracerebral</i>	Dopaminergic progenitors; neural progenitors	Stereotactic, MRI-guided delivery, with multiple deposits per tract; intraoperative confirmation of coordinates	Graft overgrowth, ectopic neural tissue, tumour formation and graft-induced dyskinesia; PET/MRI, neurological examination and motor diaries
<i>Intramyocardial</i>	Ventricular cardiomyocyte spheroids	Surgical transepical delivery using a dedicated needle/adaptor or catheter-based administration	Ectopic tissue, arrhythmogenicity, teratoma formation in the myocardium and graft loss; cardiac MRI, Holter/ILR monitoring, echocardiography and troponin/NT-proBNP
<i>Subretinal</i>	Retinal pigment epithelium	Subretinal injection of a cell suspension or sheet through vitreoretinal surgery	Graft hypertrophy, subretinal mass and epiretinal membrane; OCT, fundus autofluorescence, microperimetry and fluorescein angiography

<i>Portal / hepatic (islet)</i>	Islet-like clusters	Percutaneous transhepatic portal-vein infusion under imaging guidance	Portal hypertension, ectopic tissue, graft failure and alloimmunity; C-peptide/glucose monitoring, liver enzymes and MRI where indicated
<i>Ocular surface (corneal sheet)</i>	Corneal epithelial sheets	Surgical grafting onto the corneal surface	Graft failure, overgrowth and opacification; slit-lamp examination, in-vivo confocal microscopy and photography
<i>Intravenous / intrathecal (immune effectors)</i>	CAR-NK, CAR-T, MSC	Infusion, frequently following lymphodepletion	Secondary malignancy from edited/transduced cells, clonal expansion and persistence; haematological monitoring, bone marrow/cytogenetics where indicated, vector-persistence PCR and integration-site analysis

Published hPSC-derived cell-therapy programmes also illustrate substantial variability in the duration and structure of long-term safety follow-up. Selected examples are summarized in Table 5. These precedents are provided to illustrate different approaches to LTFU and should not be interpreted as universal minimum follow-up durations for iPSC-derived ATMPs.

Table 5. Selected hPSC-derived cell-therapy clinical-development precedents incorporating long-term safety follow-up.

Registry ID	Sponsor	Product (origin / cell type)	Indication	Phase / status	Long-term follow-up (LTFU)	Pharmacovigilance / safety-surveillance component
NCT02445612	Astellas Institute for Regenerative Medicine	MA09-hRPE (hESC-derived retinal pigment epithelium)	Stargardt macular dystrophy	Observational LTFU study / Completed	1 to 5 years post-implantation LTFU, followed by 10-year safety surveillance, up to 15 years total	Dedicated 10-year safety-surveillance protocol
NCT02463344	Astellas Institute for Regenerative Medicine	MA09-hRPE (hESC-derived RPE)	Dry age-related macular degeneration	Observational LTFU study / Completed	1 to 5 years post-implantation LTFU, followed by 10-year safety surveillance	Documented long-term risk monitoring
NCT02941991	Astellas Institute for Regenerative Medicine	hESC-RPE (retinal pigment epithelium)	Stargardt macular dystrophy	Observational follow-up / Completed	1 to 5 years after implantation	Safety and tolerability monitoring
NCT03167203	Astellas Institute for Regenerative Medicine	hESC-derived RPE cells	Macular degenerative disease	Phase 1/2 safety-surveillance / Enrolling by invitation	1-year core study + 4-year LTFU + 10-year surveillance, up to 15 years total; lifelong follow-up reported for UK subjects	Dedicated long-term safety-surveillance study
NCT04106167	Fate Therapeutics	FT500 (iPSC-derived natural killer cells)	Advanced solid tumours	Observational LTFU study / Terminated	Long-term safety and survival follow-up	Long-term safety and survival monitoring
NCT05768269	Century Therapeutics, Inc.	iPSC-derived allogeneic cell therapy (CNTY programmes)	Haematologic and solid malignancies	Observational LTFU study / Recruiting	Long-term safety and survival; late adverse-event surveillance	Dedicated LTFU protocol
NCT06477744	S.Biomedics Co., Ltd.	A9-DPC (hESC-derived A9 dopamine progenitor cells)	Parkinson's disease	LTFU study, follow-on to SB-PD-001 / Active, not recruiting	Approximately 72 months (5 years)	AESI monitoring for 5 years

NCT05897957	BlueRock Therapeutics	BRT-DA01 / bemdaneprocel (hESC-derived dopaminergic neurons)	Parkinson's disease	Observational continued evaluation / Enrolling by invitation	Monitoring through 5 years post-transplant	Long-term safety and clinical monitoring
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The 13-section transplantation protocol proposed here, together with the route-specific table, therefore treats LTFU as constitutive of the transplantation protocol rather than as a post-approval addition. Classification of the product and treatment decides this. Where an integrating, latency-capable or genome-editing element is present, gene-therapy LTFU expectations apply in full. Another determinant is the persistence of then product. Because a hiPSC derivative is a living graft, and persistence is itself a listed determinant of follow-up duration, even a non-integrating engrafting product warrants a minimum of five years of annual examination and onward contact to year fifteen. With observation outliving the trial, consent, traceability and governance must be settled before first dose.

WG7 recommendation. Transplantation protocol design and long-term follow-up (LTFU) must be fixed together. Assign every iPSC derivative a documented tier before first dose; default to Tier 2 is five years of annual examination plus contact to year 15; escalating to Tier 3 for integrating, edited or immune-cloaked grafts. Register LTFU as a sibling protocol with shared identifiers and a defined handover date, and make follow-up willingness an inclusion criterion. Pre-specify a four-class attribution taxonomy, treat tumorigenicity as an LTFU endpoint, sub-study immunosuppression withdrawal, and require bidirectional donor-to-patient traceability plus a line-level registry so signals aggregate across sponsors.

2.3.2 Therapeutic dose

Selection of the therapeutic dose for an iPSC-derived ATMP should integrate non-clinical evidence of biological activity and safety with the characteristics of the administered product, the route and site of administration and the anticipated persistence of the transplanted cells [3,6,83,101,106]. The clinically relevant dose is therefore not defined solely by the nominal number of administered cells.

For first-in-human studies, the starting dose should be scientifically justified using the available pharmacological, functional and safety data, including the expected minimum biologically active dose, the local or systemic exposure associated with the administration procedure and any product-specific safety margin [3,83,101,106]. Where dose escalation is used, the escalation scheme and observation intervals should reflect the possibility that biological effects or toxicities may emerge later than would be expected for conventional medicinal products.

Dose interpretation should also consider the composition and state of the administered product. Products with the same nominal cell number may differ in viable-cell content, proportion of therapeutically active cells, maturation state, aggregation or tissue architecture and functional activity. For structured products such as sheets, spheroids, aggregates or patches, the administered therapeutic unit and local cell density may therefore be more informative than conventional body-weight- or body-surface-area scaling [6,101].

Tumorigenicity risk should be considered in relation to dose, but total therapeutic cell number and residual pluripotent-cell burden should not be conflated. For a given residual-cell fraction, increasing the total administered cell dose increases the absolute number of residual undifferentiated cells [18,29,108]. Consequently, acceptable residual-iPSC levels and analytical sensitivity should be justified in relation to the total clinical dose, administration site and broader tumorigenicity-risk assessment. No universal residual-cell threshold can be derived solely from total administered cell number [18,29,30,108,109]. Teratoma formation is also site-dependent, and there is no universal dose threshold or cut-off. For example, Hentze et al. showed that teratoma formation depends on cell number, implantation site, matrix, host strain and observation period, with the conventional kidney-capsule assay requiring approximately 2.5×10^5 of undifferentiated hESCs [107]. However, in other tissues, significantly lower thresholds have been observed ($\sim 1 \times 10^5$ in myocardium or 1×10^4 in skeletal muscle) [108]. Some reports have describe tumorigenicity from as few as ~ 100 cells in highly sensitive tissues. Therefore, it has been concluded that no universal residual (undifferentiated) cell limit can be set and that the acceptable level must be adapted to assay sensitivity, route of administration, target tissue as well as the dose [109].

For genetically modified iPSC-derived products, the relevance of parameters such as the proportion of modified cells, transgene expression and vector-copy number depends on where the modification is introduced. Where engineering is performed at the iPSC stage followed by clonal selection, these are uniform attributes of the selected clone, established during characterization rather than dose-dependent or batch-variable specifications. Where a vector or transgene is introduced into the differentiated product, however, the modified population is heterogeneous, and these parameters become batch-variable and dose-relevant, as for primary-cell therapies. They should then be controlled and specified accordingly. In all cases, these characteristics should be considered within the product-specific safety assessment [98,105,107].

Conventional maximum-tolerated-dose concepts may not fully capture the behavior of living cellular products that can persist, proliferate, migrate or differentiate after administration [3,83,101,106]. Dose-limiting toxicities should therefore be prospectively defined according to

the product and route of administration and may include graft-related effects, immune or inflammatory reactions, abnormal proliferation or procedure-related toxicity. Absence of toxicity within a short observation window should not by itself be interpreted as identification of a maximum tolerated dose.

So far, no teratoma, dose-related malignancy or dose-limiting toxicity attributable to an iPSC or ESC-derived product has been reported in clinical trials to date. These include for example iPSC-derived dopaminergic progenitor transplantation at about 5-10 million cells or iPSC-derived cardiomyocyte transplantation [99,112].

WG7 recommendation. Starting-dose selection and dose-escalation strategies for iPSC-derived ATMPs should be justified using a product-specific integration of non-clinical efficacy, tumorigenicity and safety data, together with the intended route, anatomical site, persistence and administered product format. Dose-escalation intervals should be sufficiently informative to capture clinically relevant delayed effects before exposure of subsequent participants or cohorts.

2.3.3 Immunological considerations

The immunological strategy for an iPSC-derived ATMP should be tailored to the product and clinical context. Relevant factors include HLA compatibility, therapeutic lineage, HLA and co-stimulatory molecule expression after differentiation, inflammatory environment, anatomical site, route of administration, expected persistence, potential for retreatment and the clinical consequences of graft loss or rejection [3,6,95–97,102]. Incomplete immune evasion can shorten graft persistence, which affects both the durability of the intended effect and the interpretation of the risk-benefit balance; the required persistence differs by modality and clinical strategy, including whether repeat dosing is planned.

Allogeneic hPSC-derived products have been developed using varied approaches, including transient or sustained systemic immunosuppression, lymphodepleting conditioning, local immune protection, HLA-selected or HLA-homozygous lines and engineered hypimmune phenotypes [95–97,102]. Kirkeby et al. compiled available information of clinical trials with PSC derived products and dissected the immunosuppression information. The type, dosage and length of immunosuppression needed depend on the type of cells that are transplanted, the anatomical location, and the expected duration of graft persistence [113]. For example, retinal pigment epithelial (RPE) transplants have shown that they can survive without the need for immunosuppression while for CNS applications, like dopaminergic neurons or oligodendrocytes progenitors, 6 to 12 months immunosuppression regimes have been used. For cardiac application, 1 to 2 month transient immunosuppression was applied, while on the other hand, pancreatic islet replacement currently involves more prolonged immunosuppression or even chronic immunosuppression. For NK and T cells patients were usually given lymphodepleting conditioning prior to the infusion of the cells

While these examples show that it is feasible, they do not establish a single immunosuppression protocol that would apply to all tissues, routes and products. Where systemic immunosuppression is used, the protocol should define agents, objectives, timing, duration, tapering, prophylaxis and monitoring, rescue strategies and criteria for discontinuation or re-initiation [3,101].

The immunosuppression protocol design should be referred to as part of the investigational treatment and not as background supportive care. The protocol must specify the mechanisms of each drug, the timing in relation to infusion, the target exposure where appropriate, the intended duration, the criteria for tapering, the medications that are prohibited when used together, and how missed doses are to be managed. In some early phase situations a single calcineurin inhibitor agent may be enough, while in other cases other products will need

induction or combination therapy. These decisions should be based on relevant transplantation experience, product-specific models and emerging clinical data and should not be transferred over from solid organ transplantation. The eligibility criteria must take into account the vaccination and clinical history of the patient. Baseline testing should correspond to the regimen and to the local epidemiology. Antimicrobial prophylaxis should be based on the intensity and the expected duration of the chosen regimen as well as on relevant local guidelines.

Once the iPSC-product has been administered, immunomonitoring of the patients is critical for evaluating the immunosuppression effectiveness. Blood samples are collected at regular intervals to measure immune cell counts and antibody levels. Standard lab tests are used to check for infections, inflammation, etc. Participants also have regular check-ups to report symptoms and adverse effects. All results have to be strictly recorded and reviewed by the clinical team.

The monitoring process should be able to identify drug toxicity, infection, alloimmune injury, disease progression and adverse events related to the product. Routine safety assessments should be combined with product-specific indicators of graft survival and function. If this is supported by scientific evidence, immune monitoring may involve donor-specific anti-HLA antibodies, cellular alloreactivity, lymphocyte subsets, inflammatory markers and anti-product responses. The timing of the assays and the thresholds for interpretation should be set in advance; exploratory immune biomarkers must not be treated as validated diagnostics for rejection. Serum and peripheral blood mononuclear cell samples that have been stored can be used to carry out a retrospective investigation into any unexpected loss of efficacy or inflammatory events.

Immune and clinical monitoring

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Duration, tapering and long-term follow-up

The length of time for which immunosuppression is given should correspond to the anticipated period during which the graft is vulnerable and to the effects of immune-mediated loss. The conditions for stopping immunosuppression should be based on evidence of engraftment, stable function, the absence of clinically significant alloimmunity, and an acceptable safety profile. The protocol must take into account the possibility of an immune response rebounding after the immunosuppression is being tapered and it should clarify whether

immunosuppression can be resumed. Follow-up care must continue beyond the point at which immunosuppression is stopped if the cells remain, the product has been genome edited, or if there are still potential delayed risks relating to tumorigenesis, immunology, or the formation of ectopic tissue. According to FDA guidance a product-specific assessment of the risks of delayed adverse events is recommended, and genome editing is specifically cited as a reason for including long-term follow-up in clinical protocols [100]. The EMA guidance does likewise by requiring risk-based follow-up and pharmacovigilance for cell-based medicinal products [101].

The choice of an immunocompatibility strategy

In order to diminish the immunosuppression needs when using allogeneic products, different strategies regarding the choice of genetic background of the iPSC have been designed, based on avoiding allorejection due to HLA mismatch. One such strategy is the use HLA-selected or HLA-homozygous iPSC lines, which may reduce adaptive alloimmune pressure but should not be assumed to eliminate immunogenicity. Compatibility depends on the loci and typing resolution used for matching, HLA expression by the differentiated product, minor histocompatibility antigens, pre-existing or treatment-emergent donor-specific antibodies and innate immune recognition. High-resolution HLA confirmation should therefore be performed at an appropriate bank-qualification stage and linked to a defined recipient-matching strategy. HLA expression after differentiation should be characterized where relevant to clinical interpretation [95–97,102].

Another strategy is the use of Genome-editing to reduce immune recognition which include modification of B2M or CIITA (to avoid HLA-I and II expression and therefore recognition by host T-cells) and expression of immunomodulatory molecules such as CD47, HLA-E, HLA-G or PD-L1 (mainly to inhibit NK attack on cells not expressing HLA molecules). Such strategies may reduce adaptive recognition but can introduce additional concerns related to innate immune recognition, abnormal persistence, immune escape and genomic consequences of editing. Evaluation should therefore address both adaptive (T-cell) and innate (NK and Myeloid) immune interactions and should integrate the engineered immune phenotype with genomic and broader safety assessment [95–98,102,105]. Stable immunomodulatory transgenes are characteristics of the final product, whereas transient editing reagents are manufacturing-process components addressed in Section 2.2.4.

The expected benefit of reduced alloreactivity should be balanced against risks associated with immunosuppressive treatment, including infection, drug toxicity, malignancy and metabolic complications.

Recommendations for immunosuppression:

- Give a reason for the immunosuppression concerning the used drug/s, the route, the anatomical site, the HLA typing and the intended persistence.
- Treat immunosuppression as a key part of the protocol with certain drugs, goals, length, tapering, rescue treatment, and stopping rules.
- Use the regimen that involves the least intensity and which is supported by evidence specific to the product as well as being compatible with sustained efficacy.
- Set up the algorithms for infection screening, vaccination, prophylaxis, and the monitoring and management of drug toxicity.
- Combine the clinical evaluation of the graft with appropriate immune system monitoring and keep the samples on file for any unexpected immune events.
- With products that have been engineered for HLA compatibility or are hypoimmune, evaluate recognition by T cells, antibodies, and NK cells and keep an eye out for any abnormally long persistence or immune escape.

- Make sure that the immunosuppression section is coordinated with the sections on transplantation, dose, pharmacovigilance and long-term follow-up in order to prevent duplicate requirements.

WG7 recommendation. The immunological strategy for iPSC-derived ATMPs should be justified case by case and should integrate HLA compatibility, product phenotype, anticipated persistence, site of administration and the risks associated with immunosuppression or immune-evasive engineering. For HLA-homozygous haplotypes, high-resolution HLA confirmation and a defined recipient-matching strategy should be established, but HLA matching should not be considered sufficient evidence of immune compatibility. Hypoimmune products should be evaluated for both adaptive and innate immune recognition, with appropriate clinical surveillance for rejection, sensitization, abnormal persistence and immune escape.

Table 6. Immunological strategy according to iPSC-derived product type and allogeneic context.

<i>Autologous product</i>	Alloreactivity is reduced but not eliminated; manufacturing changes, neoantigens and disease related autoimmunity may remain relevant.	Assess product immunogenicity and disease specific autoimmunity; do not assume that systemic immunosuppression is unnecessary without justification.
<i>HLA selected or partially matched allogeneic product</i>	Matching may reduce adaptive immune pressure but does not remove minor antigen or innate immune responses.	Document matching algorithm and residual mismatch; predefine rescue treatment and sensitization monitoring.
<i>Unmatched allogeneic product</i>	Higher probability of T cell and antibody mediated rejection.	Justify induction and maintenance regimen, target exposure, tapering and stopping rules.
<i>Immune privileged or locally protected site</i>	Local anatomy may reduce but not abolish immune recognition, especially with inflammation or barrier disruption.	Use site specific evidence; include local imaging or functional graft assessment and systemic alloimmune surveillance where informative.
<i>Hypoimmune genome edited product</i>	Reduced HLA expression may limit T cell recognition but can increase NK cell susceptibility; additional immune evasion genes may create distinct safety risks.	Demonstrate balanced T cell, antibody and NK cell susceptibility; include surveillance for escape, persistence and abnormal expansion.

Table 7. Suggested safety and immune-monitoring domains during immunosuppression and follow-up.

<i>Drug safety</i>	Blood count; renal and hepatic function; blood pressure; glucose and drug concentrations where relevant.	Dose adjustment, temporary interruption or withdrawal according to prespecified toxicity rules.
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<i>Infection</i>	Symptoms and examination; targeted microbiology; viral monitoring when required by the regimen or risk profile.	Diagnostic workup, antimicrobial treatment and reassessment of immunosuppression intensity.
<i>Alloimmunity</i>	Clinical or functional graft readout; donor specific antibodies and cellular assays when informative.	Confirmatory assessment before escalation; distinguish rejection from product failure or underlying disease.
<i>Abnormal growth</i>	Product and site specific imaging, laboratory or functional surveillance integrated with long term follow up.	Expedited investigation and predefined access to biopsy, ablation or surgical removal when feasible.

2.4 Summary of quality, safety and clinical recommendations

The recommendations below consolidate the principal WG7 conclusions arising from the quality, safety and clinical considerations reviewed in Sections 2.1-2.3. They represent WG7 expert recommendations and should be interpreted within the product-specific, risk-based framework described throughout this deliverable rather than as universal regulatory requirements. These recommendations synthesize the regulatory and scientific evidence reviewed above [2,3,6,18,29,30,69,84,94,101].

Table 8. Summary of WG7 quality, safety and clinical recommendations for iPSC-derived ATMP development.

Concept	WG7 recommendation
<i>Identity</i>	Use product- and lineage-specific markers to demonstrate the intended therapeutic phenotype. Complementary or orthogonal methods should be used during product characterization where appropriate, while the routine release strategy should remain quantitative, practical and appropriately validated.
<i>STR/ authentication</i>	Reconfirm cell identity against the qualified iPSC starting material using an appropriate authentication strategy. Authentication may be controlled at the bank or intermediate stage rather than serving as the principal final-product identity assay.
<i>HLA</i>	For HLA-matching strategies, confirm the relevant HLA haplotype at high resolution at an appropriate stage of cell-bank qualification, maintain traceability to the administered product and define the recipient-matching strategy. Assess HLA expression after differentiation where clinically relevant.
<i>Purity</i>	Define and quantitatively control the intended therapeutic population and clinically relevant off-target populations, including residual iPSCs, poorly differentiated intermediates and other populations that may adversely affect safety, efficacy or consistency. For intentionally heterogeneous products, establish a predefined cellular composition.

<i>Potency</i>	Use a quantitative functional readout linked to the proposed mechanism of action where feasible. Where no single assay adequately captures product function, use a justified potency-assurance matrix. Distinguish routine release potency testing from development-stage functional characterization and preclinical efficacy.
<i>Viability</i>	Assess viability under conditions representative of administration, particularly post-thaw where applicable. The method should be appropriate for the final product format and may be complemented by additional viability or apoptosis characterization where justified.
<i>Cell content / administered dose</i>	Define the administered dose according to the final product format, integrating viable cell content and, where relevant, target-cell proportion, cellular composition and structural attributes.
<i>Genomic integrity</i>	Use a stage-appropriate combination of cytogenetic and molecular methods, interpreted where possible against the donor germline. Karyotyping or equivalent approaches can detect gross abnormalities, while higher-resolution methods such as SNP/CNV analysis, targeted sequencing, WES or WGS may provide complementary characterization according to product-specific risk. These can be complemented by higher-depth targeted sequencing of driver genes where relevant. Interpretation remains case-specific.
<i>Cancer-related panels</i>	Cancer-related genomic panels may provide informative characterization, but findings should be interpreted according to variant type, clonality, biological consequence and broader tumorigenicity evidence rather than as a binary safety test.
<i>Residual iPSC</i>	Validate a product-specific analytical strategy and justify assay sensitivity in relation to total administered dose, product format, implantation site and tumorigenicity risk. A sensitive molecular assay may provide the routine analytical backbone, with an orthogonal functional approach added during characterization where warranted.
<i>Tumorigenicity</i>	Use a product-specific weight-of-evidence strategy integrating residual-iPSC control, assessment of abnormal proliferative behavior, genomic characterization and, where justified, appropriately designed in-vivo studies.
<i>Reprogramming components</i>	Control primarily at the iPSC qualification stage; reconfirm at later stages when justified by the reprogramming method and residual-risk assessment.

<i>Gene-editing components</i>	Define residual-component testing according to the editing platform and manufacturing process. Demonstrate clearance at the stage most relevant to residual risk and integrate editing-related risks into clinical monitoring and LTFU where delayed risks justify it. For rapidly clearing transient reagents such as RNP, a dedicated clearance study may not be required where justified by documented clearance kinetics, clonal selection and extended culture.
<i>Sterility</i>	Use pharmacopoeial methods or appropriately validated rapid alternatives. Where complete sterility results are unavailable before administration, establish justified pre-administration controls and predefined management of subsequently detected contamination.
<i>Mycoplasma</i>	Apply an appropriate pharmacopoeial or validated molecular mycoplasma test suitable for the product matrix and intended release timeline.
<i>Endotoxin</i>	Apply an appropriate pharmacopoeial or otherwise validated endotoxin test, with limits justified for the dose, formulation and route of administration.
<i>Adventitious agents</i>	Use a risk-based strategy that identifies where contamination may be introduced and where it can be most effectively detected or prevented. Do not assume routine final-product testing where risk is more appropriately controlled at donor, cell-bank, raw-material or process level.
<i>Therapeutic dose</i>	Justify the starting dose and dose-escalation strategy using product-specific non-clinical efficacy and safety data together with the administration route, anatomical site, persistence and product format. Escalation intervals should account for potentially delayed effects.
<i>Transplantation protocols</i>	Identify plausible delayed risks from the outset and integrate route-, site- and product-specific surveillance into the clinical protocol.
<i>Immunosuppression</i>	Justify immunosuppression according to the specific product and clinical context. Use a regimen whose intensity and duration are supported by the expected immune risk and therapeutic requirements, together with appropriate immune, infection and treatment-toxicity monitoring.
<i>Alloreaction</i>	Monitor clinically relevant rejection, sensitization and other alloimmune responses. For hypoimmune or immune-evasive products, additionally monitor abnormal persistence, loss of

	immune visibility and other consequences of altered innate or adaptive recognition.
<i>LTFU</i>	Plan delayed safety monitoring prospectively from the beginning of clinical development. The duration and intensity of LTFU should be justified according to product persistence, tumorigenicity, immunological and genetic risks and the administration procedure.

3 Investigational Medicinal Product Dossier (IMPD) and a staged approach to regulatory consultation

3.1 Scope and positioning

This section is written for therapeutic developers, in academia and industry, who intend to bring induced pluripotent stem cell (iPSC)-derived products into clinical trials in the European Union (EU) and the European Economic Area (EEA). It forms part of Deliverable D7.2 of Working Group 7 (WG7, clinical application) of the HAPLO-iPS COST Action (CA21151; COST, European Cooperation in Science and Technology), a network working towards iPSC lines that are homozygous for frequent human leukocyte antigen (HLA) haplotypes (“haplotypes”) for allogeneic use. The section addresses three practical questions: (i) **what the Investigational Medicinal Product Dossier (IMPD) should contain**, the file of quality, non-clinical and clinical data that supports a clinical trial; (ii) **in what order, and with which agency, regulatory consultations should be sought**; and (iii) which practical tools, in the form of checklists and templates, developers can use to prepare (Section 3.5). The IMPD supports a first-in-human (FIH) clinical trial application (CTA), that is, the application to the competent authorities and ethics committees for authorisation to conduct a clinical trial.

3.2 iPSC line starting material, GMP and master files

3.2.1 Starting material and GMP expectations

The iPSC line from which a product is manufactured is treated as the **starting material** of the iPSC-derived ATMP, rather than as a medicinal product [1]. The EMA guideline expects a characterised master cell bank (MCB), that is, a uniform, cryopreserved stock of the cell line from which all production derives, and, where possible, a working cell bank (WCB) prepared from the MCB. The banks should be generated and tested according to the guideline of the International Council for Harmonisation (ICH) Q5D [44] and the relevant texts of the European Pharmacopoeia (Ph. Eur.), and the MCB should be established before exploratory trials [2]. For iPSC-based investigational ATMPs, the EMA guideline states that phase appropriate GMP and its own scientific recommendations **apply after procurement of the cells, including iPSC generation and the subsequent selection process** [2]. It recognises that cell material is limited at the earliest steps and that, where iPSC banks were generated before a clear product concept existed, oversight and documentation may have been less stringent; in that exceptional situation, the GMP principles should at minimum have been followed, as described in section 7.35 of the EU GMP guidelines for ATMPs [2,45]. Some deficiencies, in particular donor eligibility, consent for the intended use and donor-to-bank traceability, may

not be remediable retrospectively and can preclude clinical use. Section 7.35 accepts initial steps performed outside a GMP environment only exceptionally and only where a risk analysis defines the testing needed to assure the quality of the starting material. It places overall responsibility for quality, and for its impact on safety and efficacy, on the ATMP manufacturer or sponsor, even where activities are outsourced; it requires release of the material by the person responsible for quality control; and it states that the competent authorities should agree the control strategy during assessment of the CTA or marketing authorisation application [45]. A line derived outside a full GMP framework is therefore not automatically excluded, but the deviation must be justified and risk-assessed in the IMPD (Table 11). Some deficiencies, in particular donor eligibility, consent for the intended use and donor-to-bank traceability, may not be remediable retrospectively and can preclude clinical use.

Developers who obtain an iPSC line from a third party, such as a haplobank, should hold the following documentation, each element of which is grounded in the EU GMP guidelines for ATMPs [45]: (a) the derivation history of the line, which sponsor and manufacturer should communicate exhaustively to each other (sections 1.18 and 1.19); (b) a written agreement with the supplier and verification that supplied materials meet the agreed specifications, proportionate to risk (section 7.25 and section 13 on outsourced activities, which also provides for audits and inspections by the contract giver and the competent authorities); (c) evidence of GMP compliance, in which the Qualified Person (QP), the person who certifies ATMP batches before release for use in a clinical trial, may rely on third-party audits provided that responsibilities are clearly delimited and the audit documentation is accessible (sections 11.11, 11.20 and 11.21); and (d) donor-to-recipient traceability. In this respect, Regulation (EU) 2024/1938 on substances of human origin (SoHO), which will replace Directive 2004/23/EC on donation, procurement and testing of human cells from 7 August 2027, is relevant to procurement of the starting cells [46]. How the iPSC-producing step is regarded by the national competent authority (NCA), the national medicines regulator of the Member State concerned, should be confirmed during national scientific advice (Section 3.4), consistent with the expectation in section 7.35 that the competent authority agrees the control strategy.

3.2.2 Master files

A master file is a confidential dossier in which a manufacturer or supplier submits detailed information on a material, process or facility directly to a regulatory authority. Applicants that use the material can refer to the file in their own applications without receiving its confidential content, while the authority assesses it together with the application. In the United States, this role is served by the Food and Drug Administration (FDA) Drug Master File (DMF): a submission that provides confidential, detailed information about facilities, processes or articles used in manufacturing; it is neither approved nor disapproved in its own right, but is reviewed when an application refers to it, for example an Investigational New Drug application, under a letter of authorisation from the DMF holder [47].

The EU counterpart is the Active Substance Master File (ASMF). Its objective is to protect the confidential know-how of the active-substance manufacturer while the applicant remains fully responsible for the quality of the medicinal product. The ASMF is divided into an Applicant's Part, containing information that the holder regards as non-confidential to the applicant, and a Restricted Part, which is confidential and available to the authorities only [48]. A **Certificate of Suitability to the monographs of the European Pharmacopoeia (CEP)** is a certificate issued by the European Directorate for the Quality of Medicines & HealthCare (EDQM) confirming that the quality of a substance is controlled by the relevant Ph. Eur. monograph [49].

Under the EMA guideline, reference to an ASMF or a CEP is neither acceptable nor applicable where an investigational ATMP contains biological or biotechnological components in addition to the cells; the only accepted reference to a CEP concerns the TSE status of materials used in manufacture [2]. The guideline does not describe a master-file route for cell-based starting materials such as an iPSC line. The European Commission's proposed Biotech Act (December 2025) would allow ASMFs and CEPs for ATMPs [50], but it remains in the ordinary legislative procedure and adoption is not expected before the end of 2026 at the earliest [51]. At present, developers should therefore include the complete starting-material data in section S.2.3 of the IMPD. Whether a supplier's confidential dossier on an iPSC line can be cross-referenced by letter of access in a CTA is not addressed in the sources reviewed and should be raised in scientific advice.

WG7 proposes, as a preparatory measure, that haplobanks and other suppliers of iPSC lines compile a standardised iPSC Starting Material Dossier, structured on the S.2.3 headings of the EMA guideline (Table 9). A developer could incorporate such a dossier into its IMPD under confidentiality and quality agreements, and it could later be converted into an ASMF should the legislation permit.

3.3 Guidance on IMPD content for an iPSC-derived product

The IMPD is submitted, together with the trial protocol and the Investigator's Brochure (IB), the compendium of clinical and non-clinical data on the investigational product, in Part I of the CTA through the Clinical Trials Information System (CTIS), the single entry point for the submission and assessment of clinical trial applications in the EU/EEA, established under Regulation (EU) No 536/2014 [52]. Part II of the CTA covers aspects assessed nationally, such as informed consent and the compensation of subjects [52]. The EMA guideline organises the quality documentation as **S** (active substance), **P** (investigational medicinal product) and **A** (appendices: facilities and equipment, adventitious-agent safety evaluation, excipients), followed by non-clinical and clinical documentation [2].

Requirements depend on the stage of development. Quality data are expected to reflect increasing knowledge of the process; even for exploratory trials, where data are inherently preliminary, process parameters, in-process controls and release specifications with acceptance criteria must be documented, only methods confirmed to be suitable for their intended use should be applied, and a justification of the specification is required already for an exploratory trial. Confirmatory trials should use a manufacturing process that is as mature as feasible [2]. Under the risk-based approach [53], omissions can be justified, and where a relevant non-clinical study cannot be performed the EMA guideline accepts a comprehensive risk assessment with mitigation measures [2]. Table 1 summarises the recommended content for an iPSC-derived product.

Table 9. Recommended content of the IMPD for an iPSC-derived, cell-based ATMP (structure follows the EMA guideline [2]).

IMPD section	iPSC-specific content to provide	Key references
S.1–S.2.3 <i>Starting materials</i>	Definition of the starting materials with a risk-based rationale; donor eligibility, consent and traceability; reprogramming method, derivation history and clearance of reprogramming vectors (GAI: episomal plasmids at ≤ 1 copy per 100 cells, shown in seed and master banks [4]). MCB (and WCB where established): passage number, culture regime, raw materials. Minimum test panel recommended by GAI for clinical-grade lines [4]: identity by short tandem repeat (STR) profiling; mycoplasma, bacterial and fungal sterility, endotoxin and risk-based viral testing; karyotype by G-banding (≥ 20 metaphases); at least two pluripotency markers by flow cytometry ($>70\%$ of cells positive); differentiation to the three germ layers; single nucleotide polymorphism (SNP) array and whole-exome or whole-genome sequencing (WES/WGS) screened against cancer-gene panels, recorded “for information”. Supplier agreement and audit evidence (Section 2.1).	EMA guideline; ICH Q5D; GAI 2018

IMPD section	iPSC-specific content to provide	Key references
S.2.2–S.2.3 <i>Raw materials and genome editing</i>	Tabulated raw materials with supplier and step of use; grade, human or animal origin, TSE and adventitious-agent risk assessment (Ph. Eur. general chapter 5.2.12 on raw materials for cell-based and gene therapy medicinal products; the same principles apply to critical raw materials used to generate iPSCs). The viral-safety principles of ICH Q5A(R2) may be applied to biological starting and raw materials, although cell therapies fall outside its scope [54]. If genome-edited: editing tools, off-target analysis and residual editing components (for example guide RNA or Cas9 protein) as process-related impurities. If the product consists of or contains genetically modified organisms (GMOs), compliance with GMO legislation (Directives 2001/18/EC and/or 2009/41/EC) is required.	EMA guideline; ICH Q5A(R2); EMEA/CHMP/BWP/4 73191/2006
S.2.4–S.2.6 <i>Process control and development</i>	Differentiation protocol, critical process parameters, in-process controls, batch definition (number of MCB or WCB vials per batch), hold times and development history. Comparability plan, stepwise (analytical comparison, then extended characterisation, then non-clinical or clinical bridging only where needed), covering process changes and, for haplobank products, a switch between iPSC lines [55,56].	EMA guideline; EMA Q&A on comparability; ICH Q5E; D4.2 §5.2, §6.3
S.3 <i>Characterisation and impurities</i>	Identity and phenotype of the differentiated cells (for stem cells, markers of pluripotency, lineage commitment or differentiation state may be appropriate). Residual undifferentiated iPSC: assay principle, development, limit of detection (LOD) and limit of quantification (LOQ), spiking-based validation, and a limit justified by tumorigenicity data, assay validation. Lineage impurities; genetic stability by orthogonal methods (karyotype, SNP and copy-number analysis, targeted sequencing) at MCB and end of production; HLA typing and expression after differentiation; product- and process-related impurities.	EMA guideline; ISSCR 3.3.2; D4.2 §6

IMPD section	iPSC-specific content to provide	Key references
<i>S.4–S.5 Specifications and methods</i>	Release panel (identity, purity, viability, potency, sterility, mycoplasma, endotoxin, residual iPSC - against the limit established in S.3). Justification of the specification, required already for exploratory trials; only methods confirmed suitable for their intended use, with full validation expected later; a potency matrix linked to the mode of action, developed early because it becomes the release and comparability tool; reference materials.	EMA guideline; ISSCR (release criteria)
<i>S.6–S.7, P, A Finished product, stability, safety</i>	Composition and excipients (for example cryoprotectant), dose formulation, container and delivery device (combined ATMP if the device is integral), cryopreservation and post-thaw viability, stability and in-use stability; facilities and equipment; adventitious-agent safety evaluation; batch certification by the QP.	EMA guideline; EU GMP for ATMPs C(2017) 7694
<i>Non-clinical</i>	Before human exposure, at minimum: proof of concept (PoC) in a relevant model, support for the route and procedure of administration, the selection of a safe and biologically effective starting dose, and appropriate safety data. Biodistribution, persistence and clearance. Tumorigenicity and oncogenic potential (residual iPSC, genomic instability). Ectopic tissue formation. Immunogenicity and immunotoxicity, considered case by case (for example allogeneic HLA mismatch, and natural killer (NK)-cell “missing-self” responses for HLA-engineered lines).	EMA guideline §5; ISSCR 3.3.2.2–3.3.2.3; D4.2 §6.2, §7
<i>Clinical (protocol, IB)</i>	Benefit–risk assessment; FIH design (dose escalation, staggered administration with a waiting period informed by the expected immune response); starting-dose justification; immunosuppression regimen; monitoring for tumour formation, immune reactions (donor-specific antibodies, allo-sensitisation) and engraftment where feasible; stopping rules; long-term follow-up (LTFU) starting with the first patient, within the trial or in a registry.	EMA guideline §6; ISSCR 3.4.6.2; D4.2 §8

Two points warrant early regulatory discussion. First, regarding tumorigenicity, the EMA guideline states that the risk of tumour formation needs to be addressed before human exposure, that lifetime rodent carcinogenicity studies are usually not required, and that the package should rest primarily on in vitro and ex vivo analyses, supplemented by in vivo data where necessary [2]. The ISSCR Guidelines state a more conservative view; that all stem cell-derived products should be tested for tumorigenicity, that long-term animal studies are necessary to show that residual undifferentiated cells do not result in tumours, that spiking the largest feasible dose with the highest number of undifferentiated cells that might be present in the human dose is an option where the human dose cannot be delivered, and that the plan should be reviewed and approved by regulators before definitive preclinical studies and clinical trials begin [3]. As standard lifetime rodent carcinogenicity studies are usually not required by EMA, but in vivo data may be requested on a risk basis, EU developers should settle this scope during scientific advice with the regulators. Second, the potency assay is often the most difficult to validate and should be aligned with the mode of action before the FIH trial.

FIGURE 1. Preparing the IMPD package: what is needed, and when

IMPD component	Before Scientific Advice #1	At FIH CTA (CTIS)	Pivotal / MAA
Starting materials / MCB R GMP status of iPSC step	Line selected; derivation history, donor eligibility and traceability documented	MCB established and released; full test panel; vector clearance; supplier audit	WCB in place; bank stability; lot-to-lot consistency
Raw materials	Materials list; grade, human/animal origin, TSE and adventitious-agent risk	Qualified suppliers; adventitious-agent risk assessment complete	Final-grade materials; supply and quality agreements
Process and comparability R Comparability (incl. haploline switch)	Process outline; stepwise comparability plan	Critical parameters and in-process controls at suitability level	Process validation; comparability shown across changes and lines
Characterisation and residual iPSC R Tumorigenicity design	Residual-iPSC assay concept; genomic-stability strategy	Residual-iPSC assay with defined LOD; orthogonal genetic-stability data	Validated assays; final limits justified
Specifications and potency R Potency matrix	Potency matrix linked to mode of action; provisional specifications	Provisional specifications; methods suitable (specificity, sensitivity)	Validated methods; justified specifications; potency as release and comparability tool
Finished product and stability	Formulation, cryopreservation and delivery concept	Composition, excipients, post-thaw viability; preliminary stability	Full and in-use stability; final container closure
Non-clinical R Tumorigenicity design	PoC model chosen; tumorigenicity and biodistribution designs agreed	Most data complete: PoC, biodistribution, tumorigenicity, immunogenicity, starting dose	Remaining studies as needed (EMA guideline 5.7)
Clinical / LTFU	FIH design outline; immunosuppression and LTFU concept	Protocol, IB, dose escalation, monitoring; LTFU from first patient	Pivotal design agreed in SA #2; RMP and LTFU registry

Complete / in place
Suitability level; full validation later
Can follow at a later stage
R To raise with regulators

IMPD components (rows) against three development stages (columns). Colour indicates the expected level of completion at each stage: green, complete or in place; amber, suitability level with full validation later; grey, can follow at a later stage. The “R” icon marks items to be raised with regulators. Stage assignments are indicative and based on the EMA guideline; they should be confirmed in scientific advice. **Abbreviations:** CTA, clinical trial application; CTIS, Clinical Trials Information System; EMA, European Medicines Agency; FIH, first-in-human; GMP, Good Manufacturing Practice; IB, Investigator’s Brochure; IMPD, Investigational Medicinal Product Dossier; iPSC, induced pluripotent stem cell; LOD, limit of detection; LTFU, long-term follow-up; MAA, marketing authorisation application; MCB, master cell bank; WCB, working cell bank; PoC, proof of concept; RMP, risk-management plan; SA, scientific advice; TSE, transmissible spongiform encephalopathy; haploline, iPSC line homozygous for a frequent human leukocyte antigen (HLA) haplotype.

3.4 A staged approach to consultation with regulators

Regulatory interaction should be planned as a work-stream with its own timeline and budget, rather than as a single event. The sequence proposed by WG7 is summarised in Figure 2 and Table 10.

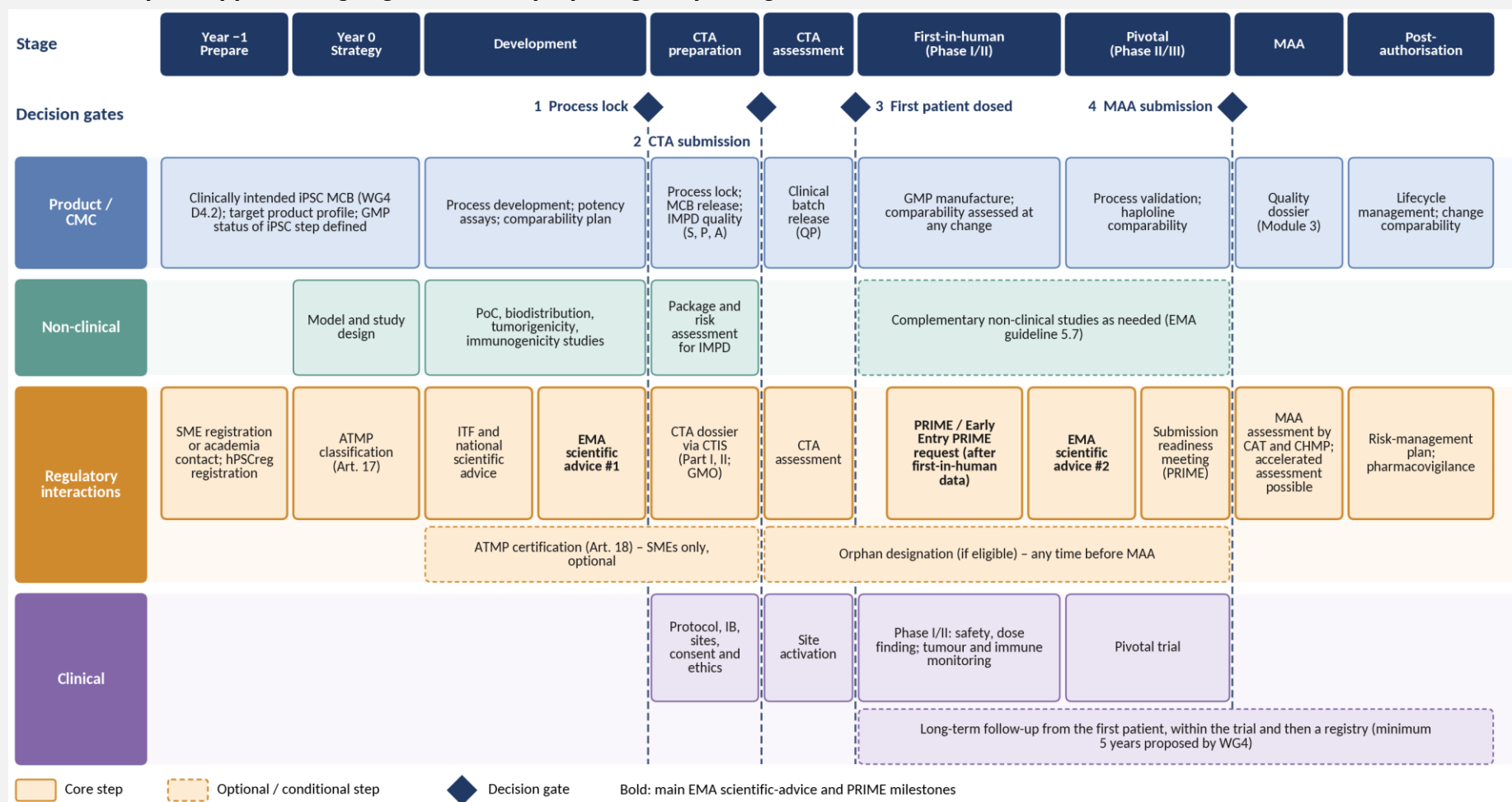
1. **Prepare (before Year 0).** Draft a target product profile (TPP; the intended indication, product characteristics and clinical use) and a regulatory strategy document, and run a gap analysis against the EMA guideline [2]. Register the starting cell line in the human pluripotent stem cell registry (hPSCreg), as recommended in D4.2 [1]. Developers that qualify as small and medium-sized enterprises (SMEs) should register with the EMA SME office, since EMA offers fee reductions for ATMP developers, larger for SMEs [57]. Applicants from the academic sector should contact EMA before submitting an eligibility request for the EMA Priority Medicines (PRIME) scheme [58] and may use the Academia briefing meetings offered by EMA [59].
2. **Confirm classification (Year 0).** Request the scientific recommendation of the Committee for Advanced Therapies (CAT), EMA's committee for ATMPs, under Article 17 of Regulation (EC) No 1394/2007. The procedure is voluntary and free of charge, and the recommendation is delivered within 60 days after consultation with the European Commission [60]. It confirms the ATMP type; for genetically modified cells, the gene therapy definition takes precedence [2].
3. **Early informal dialogue.** For questions without existing guidance, an EMA Innovation Task Force (ITF) briefing meeting offers an informal, non-binding discussion of about 1.5 hours with EMA experts, free of charge [61].
4. **National scientific advice** from the NCA of the Member State in which the CTA is planned, addressing the regulatory status and release of the iPSC line, the control strategy for the iPSC-producing step (section 7.35 of the EU GMP guidelines for ATMPs [45]), GMO requirements, and trial design. EMA also describes a pilot procedure for simultaneous national scientific advice (SNSA) from several NCAs, with guidance on the briefing book [62]; its current availability should be checked with EMA.
5. **EMA scientific advice #1**, before the process and the non-clinical package are locked. The request is submitted through EMA's IRIS online platform with a briefing document prepared on the mandatory CHMP (Committee for Medicinal Products for Human Use) template, whose core is the applicant's questions and the applicant's position on each [59] (Table 12). Priority questions for iPSC-derived products are: (a) the acceptability of an MCB generated under GMP principles before a product concept existed; (b) the tumorigenicity strategy, including the duration of any in vivo study and the sensitivity required for residual-iPSC detection; (c) comparability across haplotypes and the HLA-matching strategy; (d) the potency matrix; (e) genomic-stability testing and the interpretation of WES/WGS variants; (f) immunosuppression and immunogenicity monitoring; (g) the duration and format of LTFU, for which the EMA guideline explicitly invites scientific advice on the protocol [2]; and (h) GMO status.
6. **CTA submission through CTIS.** Part I is assessed by a reporting Member State together with the other Member States concerned, and Part II by each Member State [52]. For ATMP trials, the reporting Member State may extend the Part I assessment by a further 50 days for expert consultation (Article 6(7) of Regulation (EU) No 536/2014)

[52]; the Biotech Act proposal would remove this extension [50]. Information on national requirements for clinical trials with GMOs is available from the European Commission [2].

7. **During and after the FIH trial.** Any sponsor in the exploratory clinical phase can request eligibility for PRIME on the basis of preliminary clinical evidence of proof of concept; academic and SME applicants can request Early Entry PRIME on the basis of compelling non-clinical data in a relevant model (proof of principle) together with FIH data showing adequate exposure and tolerability [58]. Early Entry PRIME provides a scientific coordinator, an EMA product team, an introductory meeting and iterative scientific advice; the appointment of a CHMP or CAT rapporteur, a kick-off meeting and a submission-readiness meeting about one year before the marketing authorisation application (MAA) follow with full PRIME [58]. EMA scientific advice #2 should be obtained before pivotal studies (process validation, comparability, potency, pivotal design, dose).

Forthcoming instruments. The EU pharmaceutical legislation on which political agreement was reached on 11 December 2025 provides for a regulatory sandbox, to be established in consultation with Member States, in which innovative medicines that cannot be developed under existing rules may be tested under adapted requirements; it also reduces EMA's scientific committees for human medicines from five to two (CHMP and the Pharmacovigilance Risk Assessment Committee) and is expected to apply from 2028 [63]. The detailed rules have not yet been published, so developers should plan under the current framework described above.

FIGURE 2. Roadmap for approaching regulators and preparing the package



Timeline from Year -1 to post-authorisation with four swim-lanes (product/CMC, non-clinical, regulatory interactions, clinical). Diamonds mark decision gates: (1) process lock, (2) CTA submission, (3) first patient dosed, (4) MAA submission. Solid boxes are core steps and dashed boxes are optional or conditional steps; bold boxes are the main EMA scientific-advice and PRIME milestones. Box positions are indicative and do not represent durations. **Abbreviations:** ATMP, advanced therapy medicinal products; CAT, Committee for Advanced Therapies; CHMP, Committee for Medicinal Products for Human Use; CMC, chemistry, manufacturing and controls; CTA, clinical trial application; CTIS, Clinical Trials Information System; D4.2, HAPLO-iPS Working Group 4 Deliverable 4.2; EMA, European Medicines Agency; FIH, first-in-human; GMO, genetically modified organism; GMP, Good Manufacturing Practice; hPSCreg, human pluripotent stem cell registry; IB, Investigator's Brochure; IMPD, Investigational Medicinal Product Dossier (S,

active substance; P, investigational medicinal product; A, appendices); iPSC, induced pluripotent stem cell; ITF, Innovation Task Force; LTFU, long-term follow-up; MAA, marketing authorisation application; MCB, master cell bank; Module 3, quality section of the Common Technical Document; PoC, proof of concept; PRIME, Priority Medicines (EMA scheme); QP, Qualified Person; SME, small and medium-sized enterprise; WG4, Working Group 4.

Table 10. Regulatory toolbox for developers of iPSC-derived ATMPs in the EU.

Instrument	Eligibility and timing	Output	Use for iPSC-derived ATMPs
<i>ATMP classification (Art. 17)</i>	Any developer, any stage; voluntary; ideally before other procedures	CAT recommendation within 60 days; free of charge; non-binding; outcome published [22]	Confirms ATMP type (gene therapy if genetically modified)
<i>ITF briefing meeting</i>	Any developer with a question not covered by existing guidance	Informal 1.5-hour dialogue with EMA experts; free of charge; non-binding [23]	Novel questions, for example the regulatory status of the iPSC step
<i>National scientific advice</i>	Sponsor planning a CTA in that Member State	Advice of the NCA on quality, non-clinical and clinical questions; simultaneous national advice available as a pilot [24]	Control strategy for the iPSC step; MCB release; GMO requirements
<i>EMA scientific advice / protocol assistance</i>	Any stage; iterative; protocol assistance is the equivalent for orphan medicinal products [26]	Written advice on quality, non-clinical and clinical questions, based on the briefing document [21]	Tumorigenicity, potency, comparability, LTFU protocol
<i>ATMP certification (Art. 18)</i>	SMEs only; any stage with a minimum level of development	Certification of quality (and non-clinical) data in 90 days; not legally binding; data must be resubmitted at CTA or MAA [27]	De-risks the CMC package; not open to non-SME academic groups
<i>PRIME / Early Entry PRIME</i>	Preliminary clinical proof of concept; academia and SMEs may enter earlier with compelling non-clinical data plus FIH exposure and tolerability data [20]	Full PRIME: rapporteur, kick-off meeting, iterative advice, readiness meeting, accelerated-assessment potential. Early Entry: scientific coordinator, product team, introductory meeting, iterative advice [20]	After first-in-human data. Selective: 26% of requests were granted up to July 2026 [20]

Instrument	Eligibility and timing	Output	Use for iPSC-derived ATMPs
<i>Accelerated assessment; conditional marketing authorisation; orphan designation</i>	Accelerated assessment and conditional authorisation apply to the MAA; orphan designation is sought before the MAA [26]	Assessment in 150 instead of 210 days (for ATMPs in two phases of 120 + 30 days) [28]; conditional authorisation on less comprehensive clinical data for medicines addressing an unmet medical need, with specific obligations [29]; orphan: protocol assistance and 10-year market exclusivity [26]	Indication-dependent; plan evidence generation and a registry from Phase I

3.5 Practical tools for developers

The following tools translate the preceding sections into working documents. They are intended to be copied and adapted by developers, and they do not replace regulatory advice. Each entry is traceable to the cited source.

3.5.1 Tool A: documenting the iPSC line

Table 11 shows the evidence to hold in the IMPD according to how the iPSC line was derived, using the provisions of the EMA guideline and the EU GMP guidelines for ATMPs on starting materials [2,45]. Route C applies by analogy the provision of section 7.35 on cells processed outside a GMP environment.

Table 11. Evidence to hold for the iPSC line, by derivation route.

Derivation route	Evidence to hold	Basis
<i>A. Derived and banked under GMP for the intended clinical use</i>	Derivation and banking batch records; release of the MCB by quality assurance (QA); donor eligibility, consent and procurement documentation; complete test panel (Table 1); supplier agreement and audit report if produced by a third party.	EMA guideline; EU GMP for ATMPs
<i>B. Derived before a clinical product concept existed, with GMP principles followed (exceptional case)</i>	Gap analysis of the derivation against GMP principles; documented derivation history including raw materials; risk analysis defining additional testing; extended characterisation of the MCB; statement of sponsor responsibility; control strategy proposed for agreement by the competent authority; donor eligibility, consent and traceability; supplier qualification where outsourced.	EMA guideline; EU GMP for ATMPs, section 7.35

Derivation route	Evidence to hold	Basis
<i>C. Derived or isolated outside a GMP environment (research grade)</i>	Maximum available derivation and donor documentation, including donor eligibility and consent; traceability ; justification that GMP-compliant material cannot be used; risk analysis identifying the testing needed to assure quality; release by the person responsible for quality control after verification of quality and safety; control strategy agreed with the competent authority during CTA assessment.	EU GMP for ATMPs, section 7.35 (applied by analogy)

3.5.2 Tool B: template for scientific advice questions

EMA requires the CHMP briefing document template, in which questions and applicant's positions form the core of the request [59]. Table 4 is a planning aid for drafting each question before it is entered in that template; the bracketed text is illustrative and must be replaced with product data.

Table 12. Planning template for one scientific advice question.

Element	Content	Illustration (residual undifferentiated iPSC)
<i>Product and stage</i>	Product type (allogeneic or autologous, genetically modified or not), indication, stage of development, planned trial.	[Allogeneic iPSC-derived product for indication X; process locked; FIH planned]
<i>Question</i>	One decision-oriented question per topic, stating the guideline or recommendation to which it relates.	[Does the Agency agree that the proposed residual-iPSC assay, with an LOD of X, together with an in vitro and ex vivo tumorigenicity package, supports the FIH trial?]
<i>Applicant's position</i>	Proposed approach, rationale and summary of supporting data; alternatives considered.	[The applicant proposes... because... (spiking study, assay validation)]
<i>Supporting data and references</i>	Cross-references to annexes; guideline sections; published recommendations.	EMA guideline, section 5 and ISSCR Recommendation 3.3.2.2 [3]
<i>Outcome requested</i>	Agreement, comment or the additional data required.	[Agreement, or the data needed before FIH]

3.5.3 Tool C: pre-submission readiness checklist

Table 5 lists the items that a CTA for an iPSC-derived product should be able to evidence, with the document that supports each.

Table 13. Readiness checklist before submission of the CTA.

Readiness item	Evidence to hold	Basis
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☐	Regulatory route confirmed	Classification recommendation, or a documented decision not to request one; ATMP type.	Classification; EMA guideline
☐	Starting material documented	Evidence by derivation route (Table 3); MCB release; supplier agreement.	EMA guideline; EU GMP for ATMPs
☐	Raw materials controlled	Tabulated raw materials; origin; TSE and adventitious-agent risk assessment; GMO status.	EMA guideline
☐	Process and control strategy defined	Process description; critical parameters; in-process controls; batch definition.	EMA guideline
☐	Specifications justified	Release specification with justification; methods suitable for intended use; potency approach.	EMA guideline
☐	Residual iPSC and genetic stability addressed	Assay with defined LOD; orthogonal genetic-stability data, interpreted where possible against the donor germline; MCB test panel (Table 1).	EMA guideline; GAIT; ISSCR
☐	Comparability plan	Stepwise plan for process changes and for switching iPSC lines.	EMA guideline; EMA Q&A; ICH Q5E
☐	Non-clinical package or risk assessment	PoC, biodistribution, tumorigenicity and immunogenicity data; justification where a study is not performed.	EMA guideline; ISSCR
☐	Tumorigenicity plan reviewed by regulators	Scientific advice outcome obtained before definitive studies.	ISSCR 3.3.2.2
☐	Clinical protocol and IB complete	Benefit–risk; dose rationale; staggered dosing; immunosuppression; monitoring; stopping rules.	EMA guideline §6
☐	LTFU planned from the first patient	Follow-up within the trial or in a registry; duration agreed case by case.	EMA guideline §6.4
☐	Manufacturing and release arranged	GMP-compliant manufacturing site; QP certification of batches.	EU GMP for ATMPs, section 11
☐	CTA dossier assembled	IMPD, protocol and IB for Part I; national documents for Part II.	Regulation (EU) No 536/2014

3.6 iPSC-specific issues to raise early, and post-authorisation follow-up

- **Residual pluripotent cells and genomic stability:** among the principal risks of iPSC-derived products [3]; the assay, limit and validation strategy, and the methods, time

points and variant-interpretation approach for karyotype, SNP and copy-number analysis and WES/WGS, should be agreed with regulators (D4.2 §6.3).

- **Immunogenicity, allogeneic rejection and HLA-matching strategy:** the population coverage of the selected haplotypes, the immunosuppression regimen and the monitoring of donor-specific antibodies should be justified; for HLA-engineered lines, NK-cell “missing-self” responses should be addressed (D4.2 §6.2, §11.2).
- **Comparability across haplotypes:** a change of cell substrate to match a patient’s HLA type requires a demonstration of product comparability; D4.2 identifies this as potentially the most critical issue for haplobank-supported products, warranting early discussion with regulators (D4.2 §6.3).
- **Genome editing and GMO status:** whether the product consists of or contains GMOs should be determined early, since compliance with GMO legislation is then required and environmental risk assessment applies for marketing authorisation [68]; the Biotech Act proposal would exempt low-risk GMO-containing ATMPs from full environmental risk assessment [50]. For genetically modified or genome-edited products, developers should clarify the applicable regulatory classification, a reagent-specific residual editing-component strategy.
- **Long-term follow-up and post-authorisation:** EMEA/149995/2008 covers pharmacovigilance, risk-management planning (RMP) and efficacy and safety follow-up of authorised ATMPs [69]. The EMA guideline asks that patients be followed up from the FIH trial, in the trial or in a registry, with the duration agreed case by case with the regulator [2]; WG4 proposes a minimum of five years, ideally within a shared HAPLO-iPS registry (D4.2 §8). The roadmap therefore continues beyond marketing authorisation.

3.7 Recommendations from WG7

1. Treat the iPSC line as regulated starting material from the outset: apply GMP principles from derivation, document the derivation history and qualify the supplier (Section 2, Table 3).
2. Prepare a standardised iPSC Starting Material Dossier for haplotypes, ready to be incorporated into IMPDs now and convertible into an ASMF should EU law permit.
3. Sequence consultations as national advice, EMA advice #1, CTA and EMA advice #2, and use classification, PRIME and certification only where their eligibility criteria are met (Table 10).
4. Bring tumorigenicity design, potency, haplotype comparability and LTFU to the first scientific advice meeting, since these are the least standardised elements.
5. Through the HAPLO-iPS consortium, request written clarification from EMA and the national competent authorities on the regulatory status of stand-alone iPSC lines supplied to third parties, and on the acceptability of cross-referencing a supplier’s dossier.

4 Concluding remarks

iPSC-derived ATMPs represent a rapidly expanding area of regenerative medicine, with an increasing number of therapeutic programmes progressing into clinical evaluation. This growing clinical experience illustrates the translational potential of the platform while also highlighting the need for more consistent approaches to product characterization, safety assessment, clinical development and regulatory interaction.

This deliverable provides a structured assessment of quality, safety and clinical-development considerations relevant to iPSC-derived ATMPs, with particular emphasis on challenges associated with the iPSC platform. International guidelines, regulatory recommendations, peer-reviewed scientific literature and publicly available clinical-development information were reviewed and analysed, and WG7 expert recommendations were developed to support sponsors and developers in product characterization, manufacture and clinical translation.

As with other ATMPs, iPSC-derived products should be developed within an appropriate GMP framework. Their pluripotent origin and manufacturing history nevertheless introduce additional considerations, including residual undifferentiated iPSCs and associated tumorigenicity risk, genomic alterations arising during reprogramming, expansion or subsequent manufacture, complex product- and process-related impurities and, where applicable, risks associated with genetic modification. High-resolution genomic characterization is increasingly technically feasible, although interpretation of some sequence-level findings in relation to cellular-product safety remains an unresolved challenge.

The substantial diversity of iPSC-derived products, cell types, manufacturing processes, administration formats and clinical indications means that a single universal testing or clinical-development strategy is unlikely to be appropriate. A case-by-case, risk-based approach is therefore essential for defining product characterization, non-clinical development, clinical monitoring and the content of regulatory submissions. Accumulating clinical experience should progressively inform the extent and nature of these requirements, including appropriate strategies for tumorigenicity assessment, genomic characterization, immunological management and long-term follow-up.

HLA-homozygous and other HLA-selected allogeneic strategies are of particular relevance to HAPLO-iPS. Their clinical value will depend not only on the degree of HLA matching achieved but also on the differentiated product phenotype, residual adaptive and innate immune responses, recipient-selection strategy and the need for immunosuppression. Continued clinical experience will therefore be important for defining how HLA-based matching can most effectively be incorporated into allogeneic iPSC-derived therapies.

Clinical development of iPSC-derived therapies has progressed at different rates across jurisdictions, with substantial activity reported in Japan, the United States, China and an increasing number of European programmes. Differences in clinical translation are likely to reflect multiple scientific, manufacturing, financial, institutional and regulatory factors; the relative contribution of these factors remains context dependent.

To support European developers, this deliverable proposes a practical regulatory roadmap beginning with the selection and use of a clinically intended iPSC line and extending through manufacture and characterization of the differentiated ATMP, non-clinical evaluation, clinical-trial preparation, regulatory interaction, patient administration and subsequent follow-up. The roadmap is intended to complement existing ATMP guidance by highlighting issues that are particularly relevant to iPSC-derived products and by identifying points at which early regulatory discussion may reduce uncertainty.

By integrating quality, safety, clinical and regulatory considerations, WG7 aims to provide a practical reference for the development of iPSC-derived ATMPs and to support greater consistency in the evidence used to justify their clinical translation. Continued accumulation of clinical and regulatory experience will be essential for refining these recommendations and for progressively clarifying areas in which harmonized standards remain incomplete.

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Transparency statement on the use of artificial intelligence

AI-assisted tools, including Microsoft 365 Copilot, ChatGPT, NoteGPT, Claude Sonnet and Grammarly, were used during information structuring, drafting of selected sections, and language and style editing. Scientific and regulatory content was subsequently reviewed against the cited sources and revised by WG7 contributors. Responsibility for the interpretation, accuracy and final content of this deliverable rests with the authors and the HAPLO-iPS Action.

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