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Methods and reagents

Development and production of good manufacturing practice grade human embryonic stem cell lines as source material for clinical application☆

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ABSTRACT

From 2006 to 2011, Roslin Cells Ltd derived 17 human embryonic stem cells (hESC) while developing (RCM1, RC-2 to -8, -10) and implementing (RC-9, -11 to -17) quality assured standards of operation in a facility operating in compliance with European Union (EU) directives and United Kingdom (UK) licensure for procurement, processing and storage of human cells as source material for clinical application, and targeted to comply with an EU Good Manufacturing Practice specification. Here we describe the evolution and specification of the facility, its operation and outputs, complementing hESC resource details communicated in *Stem Cell Research Lab Resources*.

1. Introduction

Since the first report of pluripotent hESC isolation in 1998 (Thomson et al., 1998) there has been an aspiration to use these cells in regenerative medicine to repair disease and damaged tissue. This has driven efforts towards establishing benchmarks for the field (Andrews et al., 2005; Andrews et al., 2015), development of reagents, methods and tools to reduce or obviate the risk of transmitting adventitious pathogens (De Sousa, 2013) and the application of evolving standards of quality assurance (QA) and Good Manufacturing Practice (GMP) to satisfy regulatory aims of product safety, quality and efficacy (De Sousa et al., 2006). There have been at least two reports of "clinical grade" hESC lines compliant with US Food and Drug Administration (Crook et al., 2007; Tannenbaum et al., 2012) and numerous cell lines deposited in stem cell repositories such as the UK stem cell bank (UKSCB) designated as suitable for clinical use based on bank assessment of depositor information (go to: http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/cell_lines/approved_by_the_bank.aspx). Ultimately whether a cell line qualifies as source material for a cell therapy product (CTP) depends on its acceptance by regulatory authorities when authorisation for clinical evaluation is sought. Since cellular therapy is a relatively new science, the requirements of the regulators continue to evolve as their understanding of the issues surrounding this type of treatment has advanced. New guidance is being generated fairly rapidly, and the suitability of any cell line will be judged as and when an application is made for licensure to a particular regulatory body.

The first US FDA authorisation of an hESC derived cell product in a clinical phase 1/2a safety/efficacy (i.e. an oligodendroglial progenitor for spinal cord repair sponsored first by the Geron Corporation and then Asterias Biotherapeutics, http://www.nature.com/news/funding-windfall-rescues-abandoned-stem-cell-trial-1.15350) was founded on hESC source material first isolated using research grade reagents and laboratory conditions and subsequently transitioned into current Good Tissue Practice (GTP) and GMP. More recently, there have been several independent authorisations in the US and the EU of hESC based cell

☆ Methods and reagents
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therapy products for the treatment of variant forms of age-related macular degeneration with comparable or improved provenance (www.clinicaltrials.gov). Assessment of risk and retrospective testing, such as for adventitious pathogens, can help qualify source cell material and reagents not originally isolated under standards suitable for clinical use. However, negative results from these tests are always qualified by their limits of sensitivity. If available, source material qualified as suitable for clinical use from the onset of its derivation constitutes a preferable starting point for next generation advanced cell therapies. This is more likely to withstand elevations in the expectations of regulatory standards than non-GMP grade alternatives whose use requires more robust risk assessment.

In the EU, market authorization of Advanced Therapy Medicinal Products (ATMPs) encompassing gene, somatic and tissue engineered therapies is governed today by the European Medicines Agency (EMA) via a compulsory centralized process valid in all EU countries as well as some European Economic Area countries (Iceland, Norway and Liechtenstein). Governance is informed by European Commission (EC) directives (see Table 1) that are transacted into regulations and laws in EU member states that retain freedom to set more stringent standards or set policy regarding use of specific cell types. In the course of the effort described herein and at time of writing, the UK has developed and empowered governing bodies to regulate the procurement, processing and use of human embryo derived cells for clinical applications which aligned with EU commission directives and EMA requirements, namely; i) The *Human Fertilisation and Embryology Authority* (HFEA) for the procurement, processing, storage and use of human gametes and embryos. HFEA licensure requires that hESC lines are deposited in the UKSCB, whose terms of deposition dictate agreement to make the line available for research approved by the Medical Research Council. ii) The *Human Tissue Authority* (HTA), for the procurement, processing and storage of all human cells for human application, and iii) the *Medicines and Healthcare Products Regulatory Agency* (MHRA), as regards inspection and authorization of sites of production and application of medicines and devices such as may use cells sourced as starting materials. EU and UK authorities benefit from guidance provided by advisory committees such as for example the EMA Committee on Advanced Therapies (CAT) and UK Department of Health Advisory Committee on Safety of Blood Tissues and Organs (SaBTO). By comparison at time of writing in the US cell manufacturing processes involving substantial manipulation are deemed to be a subclass of somatic cellular therapies and regulated as biologics under section 351 of the Public Health Act and associated codes of federal regulation (see Table 2). In the US the FDA office of Cellular Tissue and Gene Therapies (OCTGT) and Centre for Biologics Evaluation and Research (CBER) authorizes/regulates investigational new drug (IND) applications for licensure of cellular and gene therapy products and associated devices, also with advisory committee input. Both jurisdictions emphasize the implementation of the highest possible quality assured practice for procurement, processing, storage and distribution, for which licensure and accreditation provide important warrants through associated inspection and audits.

Following from our prior research to derive new hESC lines under increasingly defined culture conditions (Fletcher et al., 2006) we sought to establish a facility within which our experience and evolving practice and reagents could be implemented to a GMP and professionally accredited standard. Here we describe the concurrent evolution and specification of the resulting facility established in the form of a not-for-profit company Roslin Cells Ltd, whose operation from January 2006 to November 2011, yielded seventeen new hESC lines. These were established in the course of developing and implementing QA and GMP standards in a UK authority licensed and ISO:9001 accredited facility. In so doing we believe that we have generated 8 hESC lines which comply with the current EU and US guidance and regulation governing their suitability to be considered as source material for human application. Information provided herein complements further details of resource methodology and hESC line characterisation published in *Stem Cell Research Lab Resource* format (De Sousa et al., 2009; De Sousa et al., 2016a, 2016b, 2016c, 2016d, 2016e, 2016f, 2016g, 2016h, 2016i, 2016j, 2016k, 2016l, 2016m, 2016n, 2016o, 2016p, 2016q). Cell line history files with associated protocols and records are available for auditing by prospective licensors and regulatory authorities under confidentiality agreement.

2. Methods

2.1. Overview of operational establishment

Roslin Cells (RC) Ltd was founded in October 2005 as a corporate vehicle to integrate requisite hESC science and technology, GMP cell

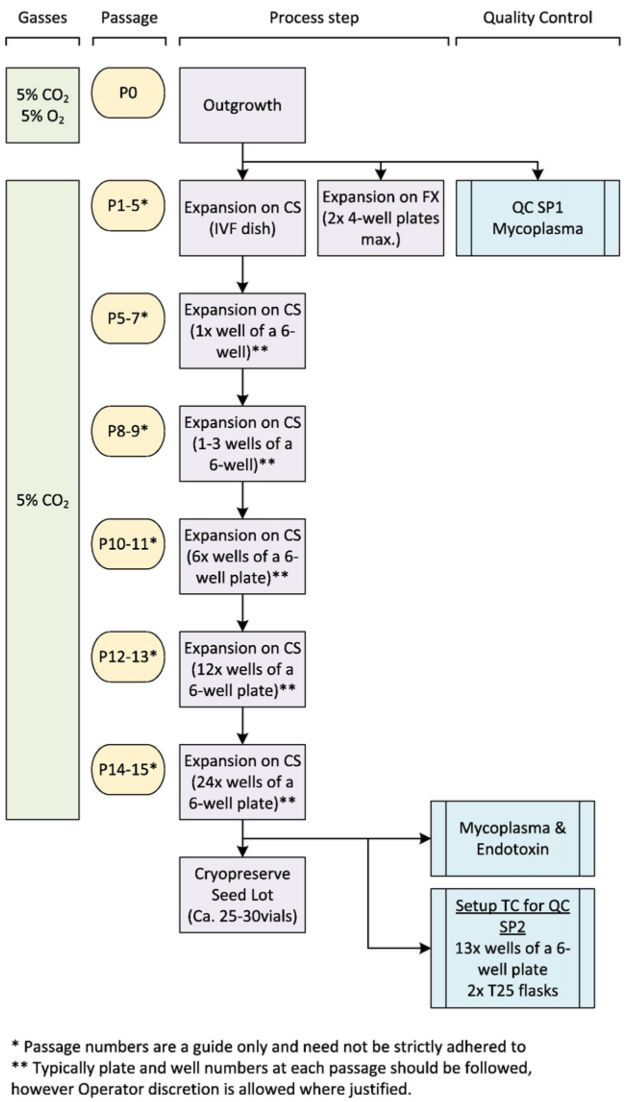
Table 1
Specification of quality assured hESC RC9, 11–17 as source material for clinical application in relation to EU Directives/Regulations governing advanced therapy medicinal products. EC – European Commission; QSS – Quality and Safety Standards; GLP –Good Laboratory Practice; GCP – Good Clinical Practice; GMP – Good Manufacturing Practice; IMP – Investigational Medicinal Product.

Phase (standard)	Directive/Regulation	Title	Compliance (☐) and/or comment (•)
Procurement (QSS)	2004/23/EC	Quality and safety standards for the donation, procurement, storage and distribution of human tissues and cells	✓ HFEA & HTA license
Processing/storage (QSS)	2004/23/EC		✓ Quality Management System.
	2006/17/EC	Technical requirements for the donation, procurement and testing of human tissues and cells	✓ Independent audits ✓ History File
	2006/86/EC	Traceability requirements, notification of serious adverse reactions and events and technical requirements for coding, processing, preservation, storage and distribution of human tissues and cells	
	2012/39/EU	Amending 2006/17/EC as regards certain technical requirements for the testing of human tissues and cells	✓ HTLV1 testing high risk donors
Clinical manufacture (GMP)	2003/94/EC	Good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use.	• RC9, 11–17 seed banks established under license from HFEA/HTA for production of source material for human application.
	EudraLex Vol 4	Guidance for good manufacturing practice for medicinal products for human and veterinary use	• Targeting EU GMP specification.
Production related issues	2009/120/EC	Amendment to 2001/83/EC relating to medicinal products for human use as regards advanced therapy medicinal products	• Traceability compliant with 2004/23/EC • Information on starting materials and developmental process as well as products
	EC 1394/2007	Regulation on advanced therapy medicinal products, amending directive 2001/83/EC and regulation (EC) No. 726/2004	• Donation, procurement, processing, storage and distribution compliant with 2004/23/EC
	EC 726/2004	Regulation laying down community procedures for the authorisation and supervision of medicinal products for human use and establishing the European Medicines Agency	• Cross-reference to MAA directive (2001/83/EC), amendments and regulation

Table 2
Specification of quality assured RC9, 11–17 hESC lines as source material for human application in relation to US FDA regulations. GLP – Good Laboratory Practice; GCP – Good Clinical Practice; GMP – Good Manufacturing Practice; GTP – Good Tissue Practice. CFR – Code of Federal Regulation; USC – United States Code (Regulation); IND – Investigational New Drug.

Process (standard)	Directive/Regulation	Title	Compliance (☐) or comment (•)
Procurement (GTP)	21CFR1271	Human cells, tissues, and cellular tissue-based products	✓ Define, document, implement, review, revise, trace procedures for traceably anonymous testing, screening, determination of donor eligibility ✓ Donor free from risk factors, clinical evidence of, infection of communicable agents using certified tests where available. ✓ Medical history assessment of risk of transmissable spongiform encephalopathy • Our donors - screened for HIV1/2, HepB/C. risk assessed and if warranted tested for communicable diseases of genitourinary tract.
Process/storage (GTP)	21CFR1271		✓ Facility environmental control, maintenance, monitoring and records ✓ Equipment procedures, calibration, inspection, records, & assurance to prevent introduction, transmission, spread of communicable diseases ✓ Supplies and reagents verified to meet specifications designed to prevent circumstances that increase risk of communicable diseases ✓ Recovery in a manner that does not introduce, transmit or spread communicable diseases ✓ Processing and process controls – verification and validation of any change in process ✓ Storage and labeling – controls to prevent mix ups, contamination, cross-contamination and assure traceability, and means for corrective actions ✓ Receipt/predistribution shipment, and distribution - controls to prevent transmission of communicable disease and safeguard product integrity ✓ Third party contract agreements ✓ Quality programme defining, documenting, implementing, reviewing, revising, auditing standard operational procedures ✓ Personnel suitability, training and competence ✓ Complaint file – establish and maintain procedures for review, evaluation and documentations of complaints ✓ Reporting – deviations, adverse events, preventative and corrective actions. ✓ Inspection ready and responsive
	21CFR1270 (Pre May 2005)	Human tissue intended for transplantation	✓ Procedures for infectious disease testing, reviewing, assessing medical records; designating and quarantining tissue, prevention of infectious diseases contamination or cross-contamination. ✓ Records – indelible and legible, identifying person performing work, documentation of tissue receipt, processing, storage and disposal ✓ Inspection ready and responsive ✓ Retention, recall, destruction competent
	42USC264	Regulations to control communicable diseases	• Not applicable – pertaining to apprehension, detection, or conditional release of individuals to prevent introduction, transmission or spread of communicable diseases from foreign countries into or within the United States
Clinical manufacture (GMP)	21CFR210	Current GMP in manufacturing, processing, packing or holding of drugs; general	• Targeting EU GMP specification.
	21CFR211	Current GMP for finished pharmaceuticals	
Non-clinical studies (GLP)	21CFR58	GLP for nonclinical laboratory studies	✓ Affirmed by BSI9001:2008 accredited.
	21CFR610	General Biological Products Standards	✓ We have established equivalence of methods, potency, sterility, mycoplasma, purity, identity, constituent materials, specifics of culture and storage, tests implemented to screen for communicable diseases.
Clinical trials (GCP)	21CFR312	Investigational new drug (IND) application	• Not applicable. IND not applied for as product constitutes source material
	21CFR50	Protection of human subjects	• Legally effective informed consent in understandable language containing basic elements of consent, without exculpatory language or waiving donor's legal rights, or appearing to release investigator, sponsor, its institution or its agents from liability for negligence.
	21CFR54	Financial disclosure by clinical investigators	✓ Embryos donated to not-for-profit organisation.
	21CFR56	Institutional review boards	• Institutional and health service for procurement of primary tissue
	21CFR11	Electronic records and signatures	• Provided by Quality Management System
Biological license application - BLA	21CFR600	Biologics	• Not applied for, but requirements consistent with specification of HTA license, Quality Management System and BSI9001:2008 accreditation.
	21CFR601	Licensing	• As for 21CFR600
	43 USC 262	Regulations of Biological products	• As for 21CFR600

manufacturing expertise and infrastructure available amongst diverse stakeholders in Scotland for the common goal of establishing resources and capability to facilitate the translation of hESC research into new regenerative medicines (Fig. 1). Founding institutions included the University of Edinburgh and Roslin Foundation (Stakeholders), and the Scottish National Blood Transfusion Service (SNBTS) and Scottish Enterprise (SE) (Observers), providing contributions in kind and funding (SE). This formed part of a greater strategic commitment of these institutions in support of the advancement of regenerative medicine ultimately including the construction of the University of Edinburgh Scottish Centre for Regenerative Medicine (SCRM; https://en.wikipedia.org/wiki/Scottish_Centre_for_Regenerative_Medicine). The facilities, operation and cell lines described herein were established in purpose built facilities constructed and operating at the Roslin Biocentre from 2005 to 2011 (Fig. 2). After this time the operation was relocated into an expanded (1000 m^2) GMP cellular therapy facility constructed within the SCRM, which at the time of writing remains the principal storage site of banked hESC lines.



Master production schedule for GMP hESC derivation and banking. Exemplar of a schematic representation of a master production schedule specifying incubator atmosphere, processing step (expansion or cryopreservation) in relation to passage number (P), vessel format (IVF petri dish vs 6 well plate), matrix (Cell Start, Invitrogen) or xeno-free feeders (FX), quality control (QC) sample points (SP), and tests performed (mycoplasma, endotoxin).

Within the organisation, key responsibilities of an executive team composed initially of a chief executive, scientific and operations officers, included corporate governance (reporting to a board of institutional stakeholders and observers), application for and renewal of UK regulatory licensing (HTA and HFEA, respectively) and professional accreditation (British Assessment Bureau – BA – ISO 9001 certification), facility design and establishment of a Quality Management System. The latter was founded on Q-Pulse software, adapted for use as guided by partners at the SNBTS with prior experience of its application in adult cell and tissue manufacture and therapy. Key internal operational tasks consisted of: i) recruitment, training and management of staff dedicated to research and development, quality control (QC), QA and cell production; ii) cell manufacturing facility design, installation, operation and performance qualification (DQ, IQ, OQ, PQ); and iii) protocol development, qualification and implementation in the production facility. Key external engagements and associated tasks included establishment and maintenance of: i) institutional advisory and regional health service ethics committee approval of information, consent and procedures for tissue procurement; ii) contractual agreements with collaborators (assisted conception units), suppliers and service providers and specification of associated technical protocols and schedules; and iii) relationships with professional forums (i.e. UK National Clinical Human Embryonic Stem Cell Forum), repositories (i.e. UK Stem Cell Bank) and prospective licensors. Operational specifications were guided by EU and US directives and guidance (Tables 1 & 2), with all tasks converging on realisation of the central aim to establish seed banks of hESC with documented history files warranting compliance of this resource to serve as source material for clinical application (Fig. 1).

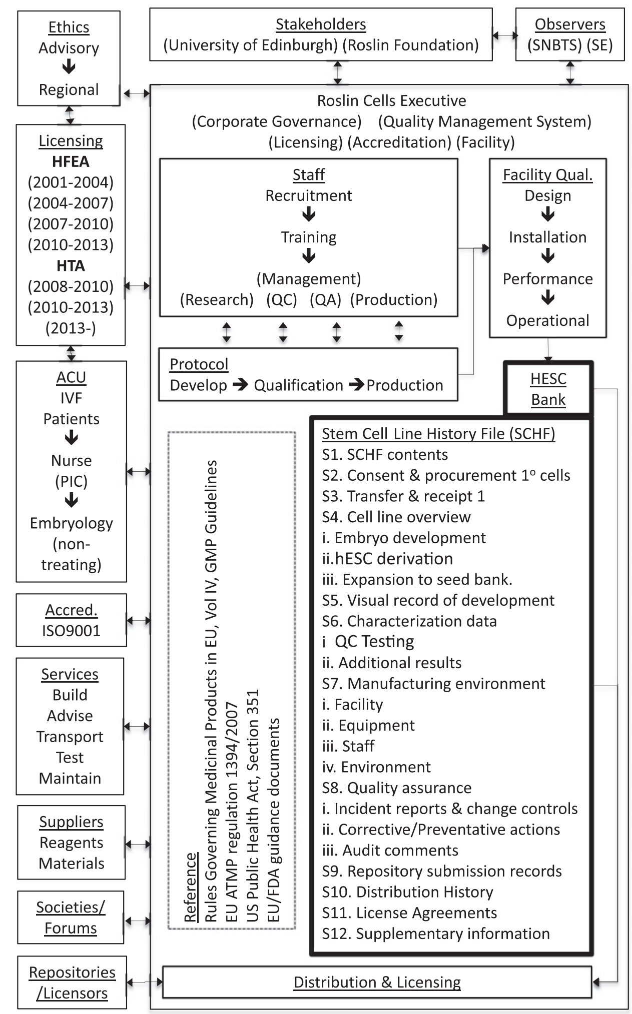
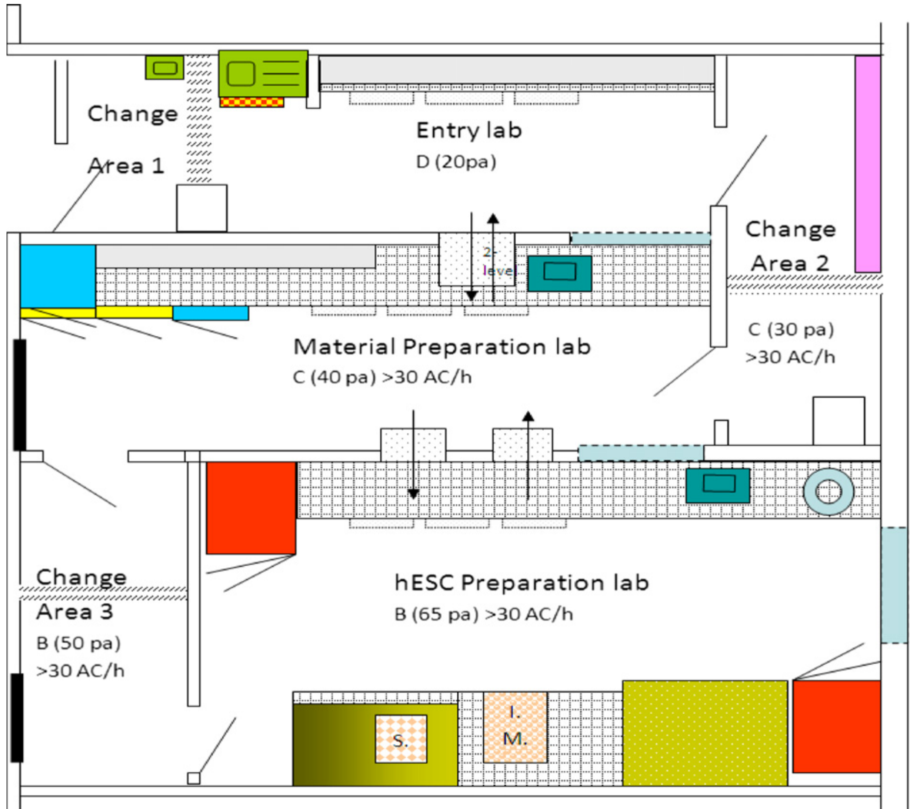


Fig. 1. Overview of intra and inter-organisational relationships in the establishment of a GMP hESC production capacity. The founding institutions of Roslin Cells included the University of Edinburgh (UEDIN), Roslin Foundation (RF), the Scottish National Blood Transfusion Service (SNBTS) and Scottish Enterprise (SE), which assumed legal status as stakeholders (UEDIN, RF) and observers (SNBTS, SE) in accordance with institutional limitations precluding conflict of interests. The organisation's executive team and staff executed responsibilities for corporate governance, quality management, licensing and accreditation and facility design, commissioning and oversight. Staff were recruited, trained and managed within separate divisions committed to research and development, quality control, assurance and cell production. Facilities underwent design, installation, performance and operational qualification. External engagements included those with institutional and regional advisory committees, UK government Human Fertilisation and Embryology (HFEA) Authority and Human Tissue Authority (HTA), Professional Standards (ISO9001) accreditation, suppliers of services and reagents/materials, repositories (UKSCB) and licensors, professional societies and forums, and assisted conception units from which embryos were procured from in vitro fertilisation (IVF) patients, whose patient information and consent (PIC) and embryo donation were administered by nurse counselors and embryologists not involved in decisions concerning patient's infertility treatment. Concurrent development of staff, protocols and facilities coupled with external engagements yielded a capability for GMP hESC manufacture resulting in banked hESC lines and auditable stem cell line history files (SCHF) available for distribution and licensing.



Room	Classification (EU GMP)	Static Pressure (Pa)	Air Change Rate (air changes/hour)
Entry (Change Area 1)	Unclassified	20	
Entry Laboratory	D	20	
Change Area 2	D/C	30	>30
Material Prep. Lab	C	40	>30
Change Area 3	C/B	50	>30
GMP Production Lab	B	65	>30

Fig. 2. Facility design and environmental specification. A purpose built controlled atmosphere facility, approximately 64 m square, was designed, constructed and validated to maintain ambient atmosphere specifications compliant with EU GMP specifications (B–D, unclassified) in order to derive and cultivate GMP grade hESC, or other human cells. This consisted of 3 laboratory spaces (HESC and Material Preparation and Entry) separated by change areas with step over barriers, and cascading air pressures, diminishing from the heard of the facility towards the entry from an unclassified atmosphere of an internal corridor of the institution (Roslin Institution) within which it was located. The air change (AC) rate throughout the facility was >30 changes/h (h), with a step change in air pressure of approximately 10–15 Pa (pa) per room from 65 to 20. Rooms were minimally equipped with horizontal and vertical flow cabinets providing an EU GMP grade A specification, light microscopes (LM), heated stages, Bench top centrifuges, stacked incubations (hESC preparation), fridges/freezers and water baths (material preparation), and moveable trolleys holding tracked disposable supplies required at time of cell processing. Materials in/out of the facility were transferred between labs via transfer hatches with cross-locking doors precluding simultaneous opening from both sides. Operator gowning in each laboratory were as required to maintain ambient atmospheric specifications.

The aforementioned governance, infrastructure, relationships and capabilities evolved concurrently (Supplementary Fig. 1). Staff recruitment, training and management commenced in January 2006 with the focus of recruitment and training shifting from research and development of hESC derivation protocols to establishment of QC/QA oversight and execution of QA cell production, as operational capacity grew and facilities were constructed. In the first instance RC occupied laboratories and office space in Roslin Institute, previously licensed by the HFEA for hESC derivation (No. R0136, centre 202, Dr Paul De Sousa, Person Responsible). Through 2007/8 we designed, installed and operationally qualified a new laboratory facility providing the controlled and graded environmental specification required for GMP cell production. Once operational this was licensed by the HFEA as well as the HTA (No. 22515, Dr Paul De Sousa, Designated Individual). This facility was first inspected by the HTA in March 2010. Concurrent with establishment of facilities and operations, hESC derivation and banking protocols underwent phases of development, qualification and finally production. Protocols varying in methodology and constituent reagents were compared against a reference standard through random allocation of donated embryos (development). Reagents and suppliers were selected on the basis of the absence or qualification of the provenance of animal or human sourced components integral to their composition or used in their production and production standards assessed by questionnaires and supplier audits. During the qualification phase, the efficacy and outcome of protocols utilising animal-free or animal/human component qualified reagents were confirmed. Confirmed protocols were then translated into a production schedule defined by comprehensive documentation of the protocol and associated information including; component reagents, batch records, staff competence, environmental specification, in process and batch release quality control parameters.

2.2. Ethical and quality assured procurement of embryos and donor screening

HFEA licensure required ethics advisory committee approval of patient information and consent and procurement procedures. This was provided by both institution specific boards (Roslin Institute/Roslin Cells and private assisted conception units (ACU) – University College Hospital) and a centralized national health service Research Ethics Committee (i.e. Scotland A) providing overarching approval for all supporting ACU, namely University College London, St Mary's Hospital (London), Aberdeen Fertility Centre, Ninewells Hospital (Dundee), and Edinburgh Assisted Conception Unit. In accordance with the first HTA and then HFEA licensing ACUs third party agreements defining mutual responsibilities were established. Embryo donor information and consent was provided/confirmed by a nurse counselor in accordance with a Standard Operating Procedure (SOP) for provision of information and consent and recording of patient information including; acknowledgement of the key terms of consent (Supplementary Fig. 2), donor screening and medical history information and records of inter-institutional shipment. All donors were screened for the tests required in Commission Directive 2006/17/EC and confirmed negative for human immunodeficiency virus (HIV-1 & 2), and hepatitis B & C, and if risk assessed by clinical carers, further tested for human T-cell lymphotropic virus (HTLVI/II, cytomegalovirus (CMV), *Treponema pallidum* (syphilis), *Chlamydia trachomatis* or Neisseria gonnorhea. During donor medical history assessment donor risks for transmissable spongiform encephalopathies, hepatitis and sexually transmitted diseases were assessed (Supplementary Fig. 3).

All embryo donations were voluntary and consisted of embryos designated as surplus fresh, surplus frozen and fresh failed for use in infertility treatment as assessed independently by embryologists involved in treatment. Surplus fresh embryos were morphologically abnormal and deemed of insufficient quality for freezing. Fresh failed embryos were a separate category of “fresh surplus” assessed to be delayed in their developmental progression and also unsuitable for use in infertility treatment. Over the 5 year period during which embryo donations were procured, a total of 548 failed, 287 surplus fresh and 270 surplus frozen embryos were utilized in the development and implementation of protocols and operational capability (Supplementary Fig. 4).

The chain of custody from ACU to RC was systematically documented. Donor identity was traceably anonymised by assignment of a tracking code. Couriered shipments were tracked and monitored live using online GPS tracking. The primary tissue was transported in specialised containers which had been validated for use. Where fresh tissue was transported the shipment was continually monitored using a calibrated temperature monitoring device. For frozen tissue being transported using a dry shipper, the temperature of the vessel was checked upon receipt as a minimum. Where possible, a dry shipper with a tracking device was used. All shipping containers were securely locked and labelled in line with regulatory requirements. Only approved courier services under third party agreements were used. Upon receipt of the shipment at RC, there were a number of checks made to ensure that the donated material fulfilled acceptance criteria, defined by RC's quality control department, independent of the derivation/production process. Checks included confirmation from the nurse counselor interfacing with the donors that consent had not been withdrawn, the receipt of pertinent documentation associated with the donation, and that the shipping container was intact. Documentation included: i) anonymised patient information and consent, ii) donor screening (including for blood borne viruses), iii) donor medical history questionnaire, and iv) a transfer documentation/checklist prepared at the ACU. Once the donation was released as acceptable for use, a unique RC tracking number was assigned to the donation for internal use. Upon receipt by the production team the shipment was checked to confirm that: i) the shipping temperature had been maintained, ii) the individual containers are intact, and iii) the numbers of units of donated material received match the numbers shipped.

2.3. Refinement of hESC derivation and banking protocols

Summary and comprehensive details of embryo and hESC provenance, culture and cryopreservation methods, reagents, environmental conditions, quality control assessment and characterization for each hESC line generated in the programme are summarized in Table 3 and Supplementary Figs. 1, 3, & 5, with further detail elaborated in corresponding Lab Resource publications (De Sousa et al., 2016a, 2016b, 2016c, 2016d, 2016e, 2016f, 2016g, 2016h, 2016i, 2016j, 2016k, 2016l, 2016m, 2016n, 2016o, 2016p, 2016q). History files for each line are available for inspection under confidential agreement. Vitrified embryos were thawed using Vitrified Embryo Safety Thawing Pack (Kitazato, Valencia, Spain) according to the manufacturer's instruction. Frozen embryos were thawed using Embryo Thawing Pack (Medicult, Måløv, Denmark) or Sydney IVF/Blastocyst Thawing Kit (Cook Medical, Bloomington, Indiana, USA) using standard techniques. Fresh or thawed embryos received prior to day 3 post fertilization were cultured in Sydney cleavage medium (Cook Medical), SAGE Quinn's Advantage Cleavage medium (Origio, Måløv, Denmark; formerly Rochford Biomedical), or EmbryoAssist (Medicult). On day 3 of development, or for embryos received or thawed after this stage, embryos were transferred to blastocyst medium for the appropriate culture system (SAGE Quinn's Advantage Blastocyst medium (Origio), Sydney blastocyst medium (Cook Medical) or BlastAssist (Medicult)). Embryos were cultured at 36.5–37.5 °C, $5 \pm 0.5\%$ CO_2, $5 \pm 0.5\%$ O_2 in drops under paraffin oil and transferred to fresh medium at least every 2–3 days. HESC derivation was initiated by whole embryo outgrowth on a supportive substrate. In the course of the programme, protocols transitioned from commencing outgrowth at 6 days post fertilization to 8 days.

The initial reference standard conditions for hESC derivation against which improvements in the protocol were compared consisted of a substrate of mitotically inactivated (by γ-irradiation) non-GMP grade human neonatal foreskin fibroblast feeders (HDF) (RCM1, RC2-8) on plasticware pre-coated with human laminin (RCM1, RC2, 3, 5–6). An extracellular matrix cocktail consisting of separately sourced human laminin, vitronectin, fibronectin and collagen IV (Ludwig et al., 2006) was used successfully to derive RC-4, but batch variation and limited supply of these reagents constrained commitment to this approach. Subsequently, it was found that pre-coating of culture vessels with laminin was superfluous for derivation and this was removed from the process from RC-7 onwards. Quality Assured GMP grade HDF approved by the US FDA for human application was licensed from Forticell Biosciences (Englewood, New Jersey, USA) and used in all subsequent derivations (RC9–17). These were cultured in medium containing pharmaceutical grade fetal bovine serum prior to mitotic inactivation (by γ-irradiation). Reference standard medium consisted of HDF conditioned Knock-Out Dulbecco Minimal Essential Medium (KO-DMEM) supplemented with knockout serum replacement (KOSR), 24 ng/ml bFGF and additional chemical nutrient supplements

Table 3
HESC lines produced in development and implementation of GMP production at Roslin Cells Ltd. 2006-2011.

Line[a]	Derivation conditions				Expansion conditions					Cryopreservation		Environment		UKSCB banking		
	Matrix		Media and supplements		Matrix		Media and supplements									
	Non-GMP HDF	GMP HDF	HDF CM KOSR	Xeno-Free GMP Media	GMP matrix	Non-GMP matrix	HDF CM KOSR	Non-Xeno Free GMP media	Xeno-Free GMP media	Non-GMP Cryo-solution	GMP Cryo-solution	Derived in GMP facility	Derived in research facility	MRC steering Approved	UKSCBMTA signed	UKSCB deposited
RCM-1	•		•		•			•		•			•	•	•	•
RC-2	•		•		•			•		•		•		•	•	•
RC-3	•		•		•			•		•		•		•	•	•
RC-4	(2)		•			•	•			•		•		•	•	*
RC-5	•		•		•			•		•		•		•	•	*
RC-6	•		•		•			•			•		•	•	•	*
RC-7	•			•	•			•			•		•	•	•	*
RC-8	•			•	•			•			•	•		•	•	*
RC-9[b]		•		•	•			•			•	•		•	*	
RC-10		•		•	•			•			•		•	•	*	*
RC-11[b]		•		•	•			•			•	•	•	•	*	
RC-12[b]		•		•	•			•			•	•		•	*	
RC-13[b]		•		•	•			•			•	•		•	*	
RC-14[b]		•		•	•			•			•	•		•	*	
RC-15[b]		•		•	•			•			•	•		•	*	
RC-16[b]		•		•	•			•			•	•		•	*	
RC-17[b]		•		•	•			•			•	•		•	*	

[a] Resource details provided in De Sousa et al. (2016a, 2016b, 2016c, 2016d, 2016e, 2016f, 2016g, 2016h, 2016i, 2016j, 2016k, 2016l, 2016m, 2016n, 2016o, 2016p, 2016q); (2) derived on mixture of laminin, fibronectin, collagen IV and vitronectin (Ludwig et al., 2006).
[b] Optimum GMP hESC as source material for production of cell therapy products for clinical application.

(Fletcher et al., 2006). The KOSR was ultimately replaced with xeno-free serum replacement and the concentration of bFGF was elevated to 80 ng/ml bFGF. For HESC expansion and banking, a feeder free system was adopted consisting of Cellstart matrix and Stempro Serum Free Medium (ThermoFisher Scientific, Waltham, Massachusetts, USA). Although most of the reagents selected for use were not formally manufactured to GMP standards, all reagents were reviewed and approved by QA as suitable for use in a GMP process. Throughout the process, cell passaging was performed mechanically by manual dissection using an EZ passage tool (ThermoFisher Scientific). Cryopreservation of hESC to establish seed banks evolved from initial use of KOSR and DMSO as cryoprotectants and unmonitored controlled rate freezing achieved by a 'Mr Frosty' isopropanol tub placed at -80 °C for 24 h to use of a GMP grade cryoprotectant (Cryostor CS10; Stemcell Technologies, Vancouver, Canada) and temperature monitored controlled rate freezing using the liquid nitrogen free controlled rate freezer EF600-107 (Grant Instruments, Cambridgeshire, UK) operated in an environmentally monitored GMP cell production facility.

2.4. Cell manufacturing environment

The GMP cell production facility was designed to comply with EU Good Manufacturing Practice Guidelines (Eudralex Volume 4), specifically for manufacture of sterile biological active substances and medicinal products for human use (Eudralex vol 4, Annexes 1 & 2). A comprehensive Site Master File has been compiled describing the establishment and operation of the RC facility in the course of the programme, and this is available for inspection under confidentiality agreement. The facility was designed to operate in compliance with EC Guide to GMP annex 1, with appropriate cleanroom design and standards of operation and monitoring. Key features of operation included regular monitoring of the facility for air temperature, humidity and pressure, and for particle and microbiological counts. Operational features included careful design of the flow of raw materials, personnel and waste, with requisite garment change areas and cross-overs. The layout and environmental specification of the facility are provided in Fig. 2. All open processing was carried out within Grade A class II safety cabinets. All hESC lines have been stored in dedicated vapour phase liquid nitrogen or mechanical freezers maintained at -150 °C $\pm$ 10 °C. These were originally at Roslin Institute and subsequently moved to the new Scottish Centre for Regenerative Medicine facility. Lines are also stored in sub-contracted off-site storage, and at the UK Stem Cell Bank (UKSCB). Mechanical freezers also feature a liquid nitrogen backup system which could deploy vapour phase liquid nitrogen to the freezer in case of mechanical failure. The ambient temperature of the storage facility is monitored to ensure that equipment is maintained as per manufacturer's recommendations.

2.5. The manufacturing process

GMP grade culture of embryos and derivation of hESC defined in production master schedules (PMSs). These documents provide process overviews and include references to all SOPs, production batch records, raw materials, equipment and consumables required. All raw materials were quarantined upon receipt until inspected by RC's QC department to ensure that only approved products, meeting the specification were used in production processes. Following procurement and culture of embryos to the blastocyst stage, zona pellucida coverings were removed mechanically if embryos had not already escaped this covering, and embryos were allowed to attach to growth substrates provided. Successful outgrowths were nurtured to form hESC colonies which were cultured until a hESC line was established. For each hESC line a seed lot of vials was cryopreserved, from which subsequent banks have been established by thawing and further expansion. Processes were appropriately segregated to minimise the risk of cross contamination. Segregation was applied to processes at different levels including the processing of donations of embryos from different donors and when processing of different cell lines. Appropriate 'line-clearance' procedures were employed between processes. All processing steps were documented in SOPs and activities were recorded in batch records. These batch records were controlled and issued by RC's QA department. All completed records were subject to a QC check and QA audit. Documentation provided traceability of all reagents, consumables and personnel involved with all processing steps.

Through-out the programme Roslin Cells sought to use raw materials suitable for GMP compliant manufacturing. The ideal source of materials are those in either of the 3 categories, i.e. licensed pharmaceutical products (such as human albumin), pharmacopoeial grade reagents (such as EP grade acids) or specific reagents which are manufactured to GMP grade. GMP grade reagents are relatively rare for use in the manufacture of cellular therapies. Where such GMP-compliant materials could not be sourced then a risk based approach was taken to assess the manufacturer, the manufacturing process and the product itself to make an informed decision on whether or not it could be used. Where possible we strived to avoid the use of products containing animal derived components with chemically defined products used as much as possible if proven to be efficacious. If animal derived products were used, then a TSE evaluation was carried out using the methodology defined in the current version of the EMA note for Guidance (EMEA/410/01). All equipment used in manufacturing was procured, validated and maintained to meet GMP requirements for the entire life cycle of the item. Equipment work files are still retained capturing a documented history for each item of equipment.

2.6. Quality control

RC established and maintains a QC department independent of production. Amongst other activities QC is concerned with inspection of raw materials, specifications and testing, maintenance of reference and archive samples of product, labeling of containers of final product, environmental monitoring and testing of operator aseptic technique. Training and testing of operator aseptic technique encompass gowning as well as technical procedures. Environmental monitoring was restricted to environmentally classified spaces of the cell manufacturing at set intervals (i.e. pre and post cleaning activities) and at the time of cell processing.

2.7. Quality control testing of hESC lines

A defined regime of quality control (QC) testing was been established for both in process assessment, batch release and additional information for hESC lines produced gathered in Certificates of Analysis (Supplementary Fig. 6, and provided in De Sousa et al., 2016e, 2016f, 2016g, 2016h, 2016i, 2016j, 2016k, 2016d for RC9, 11–17, respectively). All QC testing was performed using validated equipment by trained staff working to SOPs for each technique and recording of data. All outsourced assays were associated with formal agreements with accredited service providers.

2.7.1. Mycoplasma

Mycoplasma detection was performed using Applied Biosystems PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit and MicroSEQ™ Mycoplasma Real-Time PCR Detection Kit (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instruction. Briefly, cells were lysed and DNA isolated from the culture using a magnetic bead-based method. The purified DNA template was mixed with assay mix and Power SYBR© green master mix and run on the RT-qPCR machine. For an assay to be considered valid the discriminatory positive and extraction positive control must be "detected" and the PCR negative control and extraction negative control must be "not detected". Mycoplasma was considered detected in a sample if the cycle threshold value (Ct) was less than 35.00, the target Tm value was between 75° and 85 °C and the Tm derivative value was greater than or equal to 0.05.

2.7.2. Endotoxin

Endotoxin levels were determined using the Kinetic-QCL assay (Lonza, Basel, Switzerland) and an incubating plate reader (BioTek ELx808; Winooski, Vermont, USA) according to the manufacturer's instructions. Briefly, an unknown sample was compared with a standard curve of known levels of control endotoxin. An assay was deemed valid if the coefficient of correlation, $r \geq 0.980$ and the CV (%) for the standard curve was ≤10%.

2.7.3. Viability

Viability was determined using the Guava ViaCount assay. Briefly, the Guava Viacount reagent (Millipore, Billerica, Massachusetts, USA) containing a nuclear and a viability dye, was mixed with a single cell suspension, incubated for 5 min and analysed using the Guava easyCyte flow cytometer (Millipore). Total cell count, viable cell count and percentage viable cells were obtained.

2.7.4. Flow cytometry

A pluripotent phenotype was determined using the Human and Mouse pluripotent Stem Cell Analysis kit (Becton Dickinson - BD, Franklin Lakes, New Jersey, USA) according to the manufacturer's instructions. Oct 3/4 and SSEA-4 were included as pluripotency markers, and SSEA-1 as a differentiation marker. FITC conjugated Tra-1-60 (BD) was used as an additional pluripotency marker. Fixed and permeabilised cells were stained with the markers listed above and analysed using the Guava easyCyte flow cytometer (Millipore). Percentage expression of each marker was compared to isotype control or unstained cells.

2.7.5. Immunocytochemistry

Immunocytochemical staining was used to provide qualitative information on cell identity. hESCs were fixed using 4% paraformaldehyde (Alfa Aesar, Haverhill, Massachusetts, USA) for 20 min and permeabilised using 100% ethanol (Fisher Scientific) for 2 min. Non-specific staining was blocked using 10% donkey, goat or rabbit serum (Sigma Aldrich, St Louis, Missouri, USA) in PBS (Lonza) containing 0.01% Tween-20 (Sigma) for 1 h at room temperature. Primary and secondary antibodies at optimized dilutions were prepared in a 1% serum solution in PBS containing 0.01% Tween-20 for 2 h at room temperature or overnight at 4 °C, with unbound antibody removed by 3 × 5- to 10-min washes in PBS at room temperature. Slides were mounted in Vectashield containing DAPI (Vector Laboratories, Peterborough, UK) and stored at 4 °C in the dark prior to being viewed on a Zeiss S100 Axiovert fluorescence microscope or Nikon eC1 confocal microscope. Antibody probes consisted of those specific for AFP (1:500 mouse monoclonal IgG2a;Sigma), β-tubulin III (1:1000 mouse monoclonal IgG2b; Sigma), muscle-specific actin (1:50 mouse monoclonal IgG1κ; DAKO), Oct-4 (1:200 mouse monoclonal IgG2b; Santa Cruz Biotechnology, Dallas, Texas, USA), Nanog (1:20 goat polyclonal; R&D Systems, Minneapolis, Minnesota, USA), Tra-1-60-FITC (BD), Tra-1-81-FITC (BD), SSEA-4 (BD) (all BD sourced antibodies used at concentrations used by suppliers), Sox 2, anti-mouse IgG (1:200 goat polyclonal IgG-FITC; Sigma), anti mouse IgG (1:200, goat polyclonal Alexa Fluor 488 ThermoFisher Scientific), anti-goat IgG (1:200 rabbit AlexaFluor-488; ThermoFisher Scientific) and anti-goat IgG (1:200 donkey polyclonal AlexaFluor-594).

2.7.6. In vitro differentiation

Embryoid body mediated hESC differentiation was induced by pretreating near confluent hESC cells with 10 μM ROCK inhibitor in Stempro hESC SFM (ThermoFisher Scientific) for 1 h prior to using an EZ Passage tool (ThermoFisher Scientific) to generate even sized cell fragments. These were transferred to ultra low attachment plates (Corning Inc, Corning, New York, USA) and cultured for 7 days, replacing medium every 2–3 days. The resulting embryoid bodies were transferred into embryoid body differentiation (EBD) medium consisting of 80% KO-DMEM (ThermoFisher Scientific), 20% FBS (PAA Laboratories, Pasching, Austria), 1 mM L-glutamine, 0.1 mM β-mercaptoethanol, 1% nonessential amino acids (all ThermoFisher Scientific), plated onto glass slide tissue culture chambers (Nunc, Rochester New York, USA) coated with 0.5 % gelatin at 0.1 ml/cm^2, and cultured for an additional of 14 days, feeding every second day, before being fixed and stained.

2.7.7. In vivo differentiation

Information on the developmental potential of RCM-1, RC-9 and RC-11 to form teratomas consisting of tissues representative of all three germ layers was evaluated following transplantation under kidney capsule in adult SCID mice (RCM-1) or NOD scid gamma mice (RC-9 and RC-11). After eight weeks to three months, the animals were culled and assessed for teratoma formation. Teratomas were fixed in 4% paraformaldehyde, embedded in paraffin wax and serial sections of 7 μm thickness were cut according to standard procedures. For histological assessment, the tissue sections were dewaxed, rehydrated, stained with haematoxylin and eosin or Masson staining and mounted in DePex mounting medium. To further confirm the teratoma contained tissues derived from the three germ layers, dewaxed and hydrated serial sections were stained with Safranin O and the background stained with 0.02% aqueous Fast Green FCF. Tissue sections were analysed using bright field and microscopy and digital images were recorded.

2.8. External assays

All outsourced assays were carried out under a Quality and Technical Agreement. DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Manchester, UK) according to the manufacturer's recommendations and provided in recommended quantities to the service providers. Microsatellite PCR, or Short Tandem Repeat analysis, was used to determine cell line identity and was carried out by Public Health England. A profile was obtained for the following core alleles: vWA, D16S539, Amelogenin, THO1, CSF1PO, D5S818, D75820, D135317 and TPOX. Human Leukocyte Antigen (HLA) tissue typing was carried out by the Scottish National Blood Transfusion Service. Blood group genotyping was carried out by the Molecular Diagnostics laboratory at NHSBT. Karyotype analysis was carried out by The Doctors Laboratory or Western General Cytogenetics Laboratory (Edinburgh, Scotland). Live cells at 60–70% confluency were shipped in warm containers, fixed and analysed by standard G-banding analysis. For research grade lines, 20 spreads were analysed whereas for clinical grade lines, 30 spreads were analysed. Viral screening for cytomegalovirus (CMV), human T-cell lymphotropic virus (HTLV)-1, human immunodeficiency virus (HIV)-1, hepatitis C virus (HCV), hepatitis B virus (HBV) and Epstein-Barr virus (EBV) was carried out by The Doctors Laboratory (London, UK). European pharmacopoeia (EP) sterility testing was carried out by Moredun Scientific Ltd. (Penicuik, Scotland) using the culture method.

2.9. SNP genotyping and analysis

DNA samples were assayed using the Illumina HumanCytoSNP-12 v2.1 BeadChip. Genotyping data was initially assessed using GenomeStudio genotyping module (v1.94, Illumina). Karyostudio (v1.4, Illumina) was employed to perform automatic normalisation and to identify genomic aberrations utilising default settings of the built-in cnvPartition algorithm (3.07, Illumina) to generate B-allele frequency and smoothened Log R ratio plots for detected regions. These parameters are designed to detect CNVs greater than 75 kb and CN-LOH regions larger than 1 MB with a confidence value greater than 35. All identified regions were first cross-matched to the Database of Genomic Variants (DGV; http://dgv.tcag.ca) to identify naturally-occurring structural variations in human. CNVs that were not identified on the DGV were then checked against a list of ES cell-associated culture adaptation genomic variants published by the International Stem Cell Initiative (ISCI, Amps et al., 2011). See also Canham et al. (2015) for further details.

2.10. Quality assurance

Key reference documents informing quality assurance specifications included: i) The Rules Governing Medicinal Products in the European Union (Eurdralex) Volume IV, Good Manufacturing Practice (GMP) Guidelines; ii) UK Human Tissue (Quality and Safety for Human Application) Regulations 2007, iii) EU Tissues and Cells Directive (2004/23/EC) and related Commission Directives (2006/17/EC, 2006/23/EC and 2006/86/EC), iv) EU ATMP regulations (Regulation 1394/2007), and v) requirements for ISO 9001:2008 accreditation. A key element of a GMP compliant operation is the operation and maintenance of an effective Quality Management System (QMS). An effective QMS provides for controlled and authorised release of documentation which is periodically reviewed to adapt and meet the changing demands of the organisation and cell manufacturing processes. Significant features of the documentation system operated by RC include:

- Quality Audit Programme.
- Systems for recording follow-up and corrective action necessary when departures from authorised methods occur
- System for reporting and investigating defects and incidents.
- Quality risk management system for identifying and assessing potential hazards to minimise risk to patients, donors and the organisation.
- Risk assessment procedure used independently for decision making and planning and as part of other key QMS procedures.
- Training policy for all staff members including the principles, theory and practice of GMP.
- System for approving and releasing key materials.
- System for the compilation of batch documentation for ATMP products.
- System for validation of equipment (including IT systems), processes and QC methods.
- Change control system.
- System for product recall

3. Results

The overarching goals of the Roslin Cells programme were the development and implementation of quality assured operational management and construction of a GMP grade cell manufacturing facility centred on establishment of GMP compliant hESC derivation, expansion, cryopreservation and banking. In order to demonstrate compliance with appropriate standards, the facility was operated under ISO9001:2008 standards and licensure from the UK HFEA and HTA. In order to comply with these standards, it was necessary to compile a systematic and traceable documentation of all elements of operation from receipt of embryos until deposition of cell lines. In accordance with the concurrent development of governance and facility infrastructure, licensure, and methodology we consider that of the 17 hESC lines derived in the programme (Table 3), RC-9, 11–17 were derived to a quality assurance standard appropriate for their use as source material for clinical application. The essential attributes of these lines includes:

- A fully traceable procurement and processing
- Ethical consent, including provision for commercial use
- Detailed medical history and blood borne virus (BBV) screening of donors
- A compilation of detailed cell line history
- Clearly detailed hESC manufacturing process
- A quality control testing regime.
- A mature Quality Management System, including:

- o Regulatory inspections to obtain appropriate licenses and ensure ongoing licensing approval.
- o Provision of regular reports on the performance of the QMS and executive review.
- o Appropriate staff were in place, with effective line management, training and development.
- o Facility design, installation and operational qualification and maintenance within regulatory defined specifications.
- o Equipment procurement, performance and maintenance
- o Effective research and development to optimise the manufacturing procedures.
- o Risk assessment and adverse event response tracking.
- o Segregation of research and development, production, quality control and quality assurance operational practice.
- o Third party agreements assisted conception units and service suppliers.

4. Discussion

We describe here the development and implementation of an operation and facility in the form of a not-for-profit company, Roslin Cells Ltd, for the specific purpose of manufacturing clinical grade hESC lines for use in regenerative medicine. This yielded 17 hESC lines of which 8 lines (RC9, 11–17; De Sousa et al., 2016e, 2016f, 2016g, 2016h, 2016i, 2016j, 2016k, 2016d) are compliant with European Union (EU) directives and United Kingdom (UK) licensure for procurement, processing and storage of quality assured human cells as source material for clinical application. The production of these lines was targeted to comply with EU GMP specifications as can be confirmed through inspection of associated cell line history files and QMS controlled documentation.

The key issue for further use of these cell lines is whether or not they comply with the requirements for further processing of an ATMP. In the EU, the requirements for accepting onward processing of a master cell bank rest with an assessment with the degree of compliance with GMP. Guidance on this topic is available in a reflection paper on stem cell-based medicinal products prepared by the European Medicines Agency (EMA/CAT/571134/2009), where the key requirements for cell lines used as starting materials for medicinal products are all laid out. In addition, Annex 2 of the EC Guide to GMP provides an illustrative guide as to the level of GMP required for different stages in the preparation of a medicinal product. In this guide, donation, procurement and testing of starting tissues/cells require to have been carried out in compliance with Directive 2004/23/EC (Tissue Directive). Subsequent stages of manufacture of a clinical product require to be carried out under GMP. However, the guidance in Annex 2 makes it clear that the level of GMP adherence will increase at each stage from isolation, through MCB, WCB and towards the final product.

Our efforts were targeted to comply with an EU GMP grade specification. However, at the time of establishing seed banks, a site license from the UK MHRA for the manufacture of biologics for human application had not yet been obtained. Thus, the extent to which the lines satisfy EU GMP specifications requires retrospective inspection of cell line history files and archived QMS controlled records. Based on our understanding of EU (Table 1) directives, guidance and regulations associated with procurement, processing, non-clinical studies, and manufacture, we believe that the lines exceed specifications to serve as source material in the EU. A second generation facility established at the University of Edinburgh Scottish Centre for Regenerative Medicine has subsequently been licensed by the MHRA and the lines continue to be stored in an MHRA licensed facility. It is known that clinical trial authorisations have been granted for the manufacture of clinical products derived from cell lines which were prepared in facilities which were not operating to GMP standards. Although our first facility, in which hESC lines were derived, was not licensed by the MHRA at the time that the cell lines were manufactured, we believe that the data available would support future clinical trials and subsequent licensure of products derived from these cell lines.

Our facility was not licensed by the US FDA for the manufacture of biologics to US guidelines standards for current Good Tissue Practice. However, our interpretation of these guidelines and standards (Table 2) is that they do not differ substantially from the requirements of EC directives and of HFEA and HTA licensure. There are some differences between EU and US in terms of administration and specification of GMP. Technical specifications can also vary, such as for ambient environment classification (i.e. comparison of Eudralex Vol 4, Annex I vs FDA Aseptic processing guide for GMP). However, irrespective of such nuances, either standard provides the highest level of traceability, control and quality assurance, and aspiring to comply with either can provide a higher level of assurance of source material than otherwise is mandated by both jurisdictions currently. It is our understanding based on the analysis in Table 2 that the cell lines prepared at Roslin Cells will be suitable for onward processing in the USA. However, this will require to be tested by consultation and discussion with the US FDA. Lastly, guidelines prepared by the International Society for Stem Cell Research (ISSCR) (2006, 2008, 2016) for global standards of conduct of Human Embryonic Stem Cell Research and clinical translation originally published in 2006 and 2008 (respectively) were recently updated (see Commentary, Daley et al., 2016). Our review of these confirms compliance of all hESC lines created in our programme with applicable standards (Supplementary Fig. 7).

Author contributions

PDS wrote the manuscript. JD, KB, PD, and BT provided material contributions to the development and implementation of quality assurance, control and production and figures. PS, SD, JH contributed to the development and implementation of patient information and consent and standardised practice for liason with assisted conception units for embryo procurement. PDS, MB, and MT contributed to executive governance (as Directors) and clinical cell manufacturing (MT) and review of the manuscript.

Competing interests

Roslin Cells Ltd is a not-for-profit company limited by guarantee, for which the University of Edinburgh and the Roslin Foundation are stakeholders and the Scottish National Blood Transfusion Service (SNBTS) and Scottish Enterprise are observers. PDS, AC, and MB founded the programme/company. JD, KB, PD, and BT were employees of Roslin Cells holding positions of Chief Operating Officer, Heads of Quality Assurance and Quality Control and senior scientist, respectively, in the course of the reported work. PDS is a Principal Investigator employed by the University of Edinburgh seconded to serve as Chief Scientific Officer, regulatory license holder and executive director. MB and MT (2005–2015) served as chairman and non-executive directors of the Board of Governors (unpaid).

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Appendix A. Supplementary data

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