17 Advanced Therapies: Clinical, Non-clinical and Quality Considerations

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INTRODUCTION

Cell therapy, tissue engineering, and gene therapy, together called "regenerative medicine" or "advanced therapies" or "advanced therapy medicinal products" ((ATMPs), European union (EU) legal term), represent the most recent branch of the biotechnology revolution in medicine. These therapies constitute an innovative group of heterogeneous, research driven biopharmaceuticals, based on cells, tissues, and/or nucleic acids packaged within a viral or non-viral vector. As with many medicinal products, advanced therapies are based on ground-breaking scientific discoveries and technological advancement. With increasing knowledge of the human body's cell and tissue architecture in a healthy and in a diseased state, medical therapy became targeted. This refers not only to the metabolic, immunological, and/or pharmacological interaction but also to the more complex regeneration, repair, and replacement of human diseased tissues.

The use of advanced therapies based on live cells in medical practice is not a new concept. The first successful human hematopoietic stem cell transplantation from a healthy donor to a cancer patient took place in 1968 and is now a routine clinical procedure for bone-marrow regeneration. The true value of (stem) cell therapies was not further explored until the early 1990s when the therapeutic relevance of mesenchymal stromal cells (MSCs) was considered for the regeneration of skeletal tissue and later for broader therapeutic use. Since the turn of the millennium there has been a steady increase in the number of advanced therapy clinical trials, with a growing number of target indications. Particularly for the treatment of diseases and tissue/organ defects for which traditional therapies and medicinal products have not always provided high benefit/risk outcomes, such as Alzheimer's disease, Parkinson's disease, cancer, and muscular dystrophy, advanced therapies hold high expectations.

Currently, worldwide there are over 700 advanced therapy companies and more than 1000 active clinical trials are ongoing. However, only eight advanced therapies have been approved by the Unites States (US) Food and Drug Administration (FDA) and ten by the European Commission (EC) (December 2017; see Table 17.1) and less than a handful by other jurisdictions (e.g., Canada and Japan). The inherent complexity of these products poses unique challenges compared to other therapeutics. The manufacture of "living" materials (i.e., cells and tissues) carries great challenges in terms of consistency and process and product characterization, the latter due to the lack of (sensitive) analytical techniques. Such challenges are analogous, in many ways, to those faced in the past when the first recombinant protein biopharmaceutical products were being developed and regulated. Bringing advanced therapies to market at an acceptable cost, benefit/risk ratio, and quality has proven extremely difficult for certain products.

In this chapter we discuss the current status and unique aspects of cell therapy medicinal products, tissue engineered products, and medicinal products based on the *ex-vivo* genetic modification of cells. The latter fall legally in the category of gene therapy medicinal products. Gene therapy products involving *in-vivo* gene transfer are discussed in Chap. 16.

(DIS)SIMILARITIES WITH RECOMBINANT THERAPEUTIC PROTEINS AND OTHER BIOPHARMACEUTICALS

Although advanced therapies fall within the group of biopharmaceuticals, there are substantial differences in the area of quality/chemical, manufacturing, and controls (CMC), non-clinical, clinical, regulatory, and

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Product and classification	INN/description	Indication	Company	Approval month
Europe (EMA)				
ChondroCelect® (TEP)	Characterized viable autologous cartilage cells expanded *ex-vivo* expressing specific marker proteins	Cartilage defects of the femoral condyle of the knee	Tigenix NV	10/2009 (withdrawn 08/2016)
Glybera® (*in-vivo* GTMP)	Alipogene tiparvovec (AAV1 vector)	Hyperlipoproteinemia Type I	uniQure biopharma BV	10/2012 (withdrawn 10/2017)
MACI® (TEP)	Autologous cultured chondrocytes	Fractures, cartilage	Genzyme Europe BV	06/2013 (withdrawn 10/2014)
Provenge® (SCTMP)	Sipuleucel-T; autologous peripheral blood mononuclear cells activated with PAP-GM-CSF	Prostatic neoplasms	Dendreon UK Ltd.	09/2013 (withdrawn 05/2015)
Imlygic® (*in-vivo* GTMP)	Talimogene laherparepvec	Treatment of adults with melanoma that is regionally or distantly metastatic	Amgen Europe BV	12/2015
Holoclar® (TEP)	*Ex-vivo* autologous corneal epithelial cells including stem cells	Corneal diseases stem cell transplantation	Chiesi farmaceutici SpA.	02/2015
Strimvelis® (*ex-vivo* GTMP)	Autologous $CD34^+$ cells transduced with retroviral vector containing the adenosine deaminase gene	Treatment of severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID)	GlaxoSmithKline Trading Services Ltd.	05/2016
Zalmoxis® (*ex-vivo* GTMP)	Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)	Treatment in haploidentical haematopoeitic stem cell transplantation	MolMed SpA	08/2016
Spherox® (TEP)	Spheroids of human autologous matrix associated chondrocytes	Cartilage defects of the femoral condyle of the knee	Co.don AG.	07/2017
Alofisel® (SCTMP)	Darvadstroce; contains allogeneic expanded adipose stem cells	Treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease	Tigenix NV	12/2017
USA (FDA)				
Provenge® (SCTMP)	Sipuleucel-T; autologous cellular immunotherapy	Treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer	Dendreon Corporation	04/2010
LaViv® (SCTMP)	Azficel-T	Autologous cellular product indicated for improvement of the appearance of moderate to severe nasolabial fold wrinkles in adults	Fibrocell Technologies, Inc.	06/2011
Gintuit® (SCTMP)	Allogeneic cultured keratinocytes and fibroblasts in bovine collagen	Allogeneic cellularized scaffold product indicated for topical (non-submerged) application to a surgically created vascular wound bed in the treatment of mucogingival conditions in adults	Organogenesis Inc.	03/2012
Imlygic® (*in-vivo* GTMP)	Talimogene laherparepvec	Local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery	Amgen Inc.	10/2015

Table 17.1 ■ ATMPs approved in Europe and the USA (2008–2017)

Product and classification	INN/description	Indication	Company	Approval month
MACI® (SCTMP)	Autologous cultured chondrocytes on a porcine collagen membrane	Autologous cellularized scaffold product indicated for the repair of symptomatic, single or multiple full-thickness cartilage defects of the knee with or without bone involvement in adults	Vericel Corp.	12/2016
Kymriah® (*ex-vivo* GTMP)	Tisagenlecleucel	Treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse	Novartis Pharmaceuticals Corporation	08/2017
Yescarta® (*ex-vivo* GTMP)	Axicabtagene ciloleucel	A CD19-directed genetically modified autologous T cell immunotherapy indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma	Kite Pharma Inc.	10/2017
Luxturna® (*in-vivo* GTMP)	Voretigene neparvovec-rzyl (AAV2 vector)	Inherited retinal disease in patients who have a biallelic mutation of the RPE65 gene	Spark Therapeutics	12/2017

Table 17.1 (continued)

costs/reimbursement structure compared to recombinant proteins and other biopharmaceuticals. This is summarized in Table 17.2 and further discussed in this chapter. Therefore, the format of this chapter has a different set up as the other product group related chapters in this book.

CLASSIFICATION OF ADVANCED THERAPIES

Advanced therapies can be classified in many ways, e.g., by:

1. The therapeutic indication they aim to address, e.g., neurological, cardiovascular, ophthalmological.
2. Whether they comprise cells and/or tissues (see Fig. 17.1):
   (a) derived from and administered to the same human individual (autologous = autogeneic), hence the donor = the recipient (patient);
   (b) derived from a human (healthy) donor, who is different to the patient (allogeneic);
   (c) derived from an animal (xenogeneic; see Chap. 9); e.g., porcine islets to treat diabetes mellitus (DM)
3. The potency of the cells (i.e., omnipotent, pluripotent, multipotent, oligopotent, unipotent (see Table 17.6).
4. The *in-vivo* mode of action (i.e., pharmacological, immunological, metabolic or regenerative (i.e., regenerate, repair or replace a human tissue); see Fig. 17.2 below).
5. Their underlying technology, as described in this chapter (Mount et al. 2015):
   (a) somatic cell technologies;
   (b) cell immortalization technologies;
   (c) *ex-vivo* gene modification of cells using viral vector technologies;
   (d) *in-vivo* gene modification of cells using viral vector technologies (see Chap. 16);
   (e) genome editing technologies;
   (f) cell plasticity technologies;
   (g) three-dimensional technologies;
   (h) combinations of the above technologies.
6. The cell types (e.g., MSCs, dendritic cells (DCs), T-cells).
7. The regulatory/legislative regime applied, as briefly discussed below.

Advanced Therapies: Medicinal Products for Human Use Versus "Cell and Tissue Transplantations"

Advanced therapies often are considered biological medicinal products, a category of medicinal products for human use, meaning they are typically subject to two regulatory regimes: public health legislation and pharmaceutical legislation (see British standards institution publicly available specification (BSI PAS) 83:2012). However, some clinical interventions for cell- and tissue-based advanced therapies (*gene therapies are excluded*) are not considered being medicinal and these

Category	Characteristic	Advanced therapies	Other biopharmaceuticals
Non-clinical	Animal models	Often no relevant animal models to predict safety and particularly efficacy in humans available	Relevant animal models to predict safety/efficacy often available
	Safety testing	Tumorigenicity testing may be needed (stem cell derived products)	N.A.[a]
	ADME[b]/ pharmacodynamic studies	Often not possible/relevant	Generally performed
Clinical	Disease pathway(s) and mode of action	Often not well understood	Well understood
	First in human trials	Always in patients	Often in healthy volunteers
	PK[c]/PD[d] studies	Often not feasible/relevant	Performed
	Route of administration	Often IV[e]infusion, some times local injection, e.g., intotumor, subretinal space of eye; spinal cord; brain; intra-dermal	IV injection or infusion, SC[f], intradermal, IM[g]
	Patient monitoring	Often long-term follow-up (10-20 years)	Short term follow-up
	Track & traceability	From donor start material (tissue/cell) through manufacturing process to patient and vice versa	From starting material through manufacturing process to patient
Quality/CMC[h]	Product group	Heterogeneous	Less heterogeneous
	Type of formulation	Often a dispersion /suspension of cells	Often a solution (liquid or reconstituted lyophilizate); sometimes emulsion or suspension (vaccines)
	Dose	Mostly number of (viable) cells/kg body weight or cm^2 tissue	Usually milligram range for proteins; microgram range for vaccines; or defined as units activity/mg
	Manufacturing process	Often continuous process, no designated drug substance	Often discontinuous process, designated drug substance and drug product
		Often open and manual process steps; no platform technologies yet, automation in its infancy	Closed and mostly automated process steps; platform technologies

Table 17.2 ■ Examples of differences between advanced therapies and other biopharmaceuticals

Category	Characteristic	Advanced therapies	Other biopharmaceuticals
		Often aseptic manufacture, no sterilization possible (no viral removal and/or inactivation steps) due to viability of cell/tissue	Viral removal and/or inactivation steps; sterilization (mostly through ≤ 0.2 micron filtration)
	Batch definition	Often one batch for one to few patients; off-the-shelf products less common	Off-the-shelf (one batch for multiple patients)
	Safety	Risk for transmission of human viral infections from donor to patient; animal and human derived raw materials and excipients	Risk extremely low due to viral removal/inactivation steps; chemically defined raw materials and excipients
	Product storage and supply	Sometimes 2-8°C or room temperature - short shelf - life; often vapor phase of liquid nitrogen (at < -150°C) - longer shelf -l ife(months - years)	Mostly 2-8°C and longer shelf-life (years)
Regulatory	Landscape	Evolving regulatory landscape	Established regulatory landscape
	Guidances	Specific "advanced therapy" guidances	Guidances for biologicals and vaccines
	Classification	Product classification and product terminology not harmonized globally	Product classification and product terminology mostly harmonized globally
Ethics	Uncontrolled access to non-approved product	Stem cell tourism	Illicit use of biopharmaceuticals
	Acceptability starting material (tissue/cells)	Use of human embryos to manufacture human embryonic stem cell based product not allowed in some countries	N.A.
Reimbursement	Costs	Very high (20,000-1,000.000 Euros) per treatment	Medium-high (500-5,000 Euros)per injection

[a]N.A. = not applicable
[b]ADME = absorption, distribution, metabolism, elimination
[c]PK = pharmacokinetics
[d]PD = pharmacodynamics
[e]IV = intravenous
[f]SC = subcutaneous
[g]IM = intramuscular
[h]CMC = chemical, manufacturing, and controls

Table 17.2 ■ (continued)

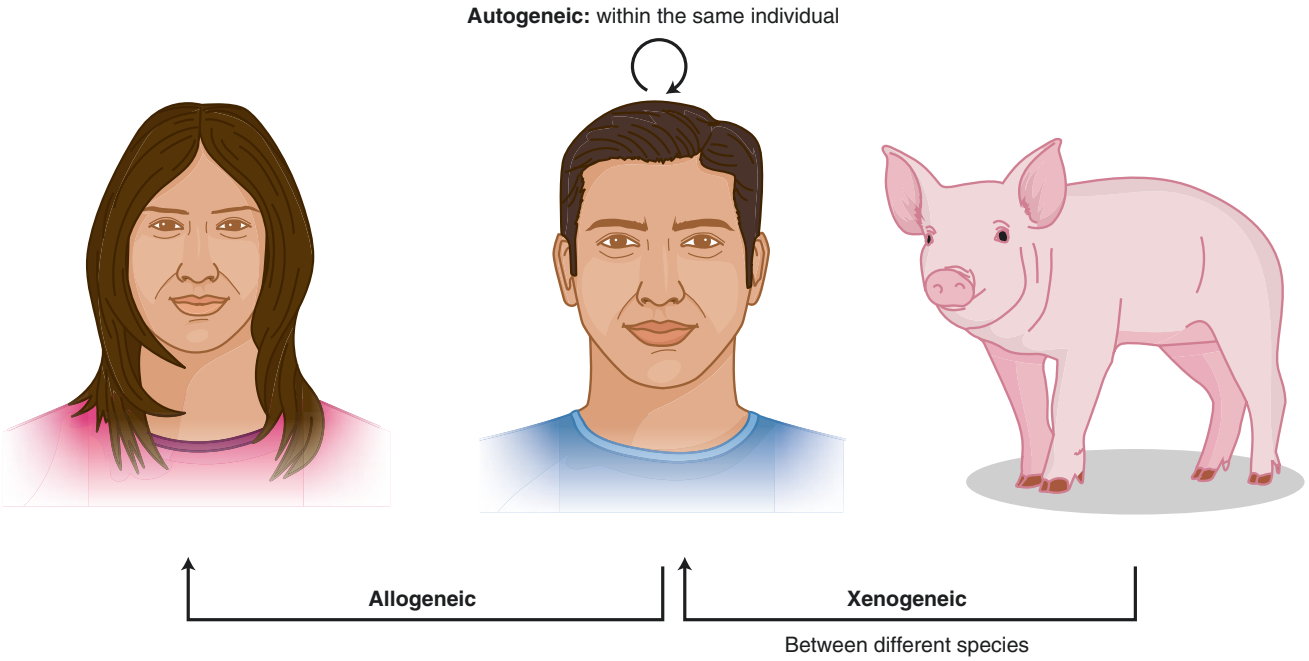

Figure 17.1 ■ Types of transplants/advanced therapy cell source

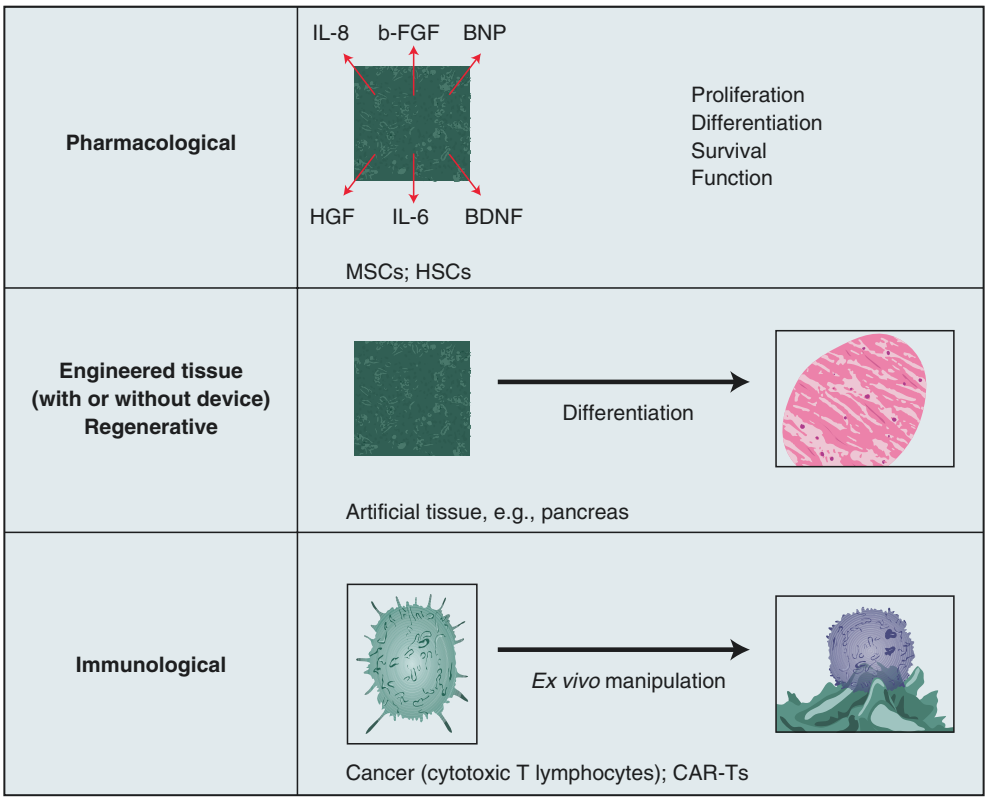

Figure 17.2 ■ Possible MoAs of advanced therapies

Substantial manipulation	Non-substantial manipulation
Specific manipulations considered substantial are: 1. Cell expansion (culture; *ex-vivo*) 2. Differentiation and/or activation with growth factors 3. *Ex-vivo* genetic modifications of cells (e.g., with viral vector)	Specific manipulations *not* considered substantial: 1. Cutting 2. Grinding 3. Shaping 4. Centrifugation 5. Soaking in antibiotic or antimicrobial solutions 6. Sterilization 7. Irradiation 8. Cell separation, concentration or purification 9. Filtering 10. Lyophilization 11. Freezing 12. Cryopreservation 13. Vitrification

Table 17.3 ■ Substantial and non-substantial manipulations; see for details PAS83:2012

therapies are subject to public health legislation only. These therapies are often called "cell and tissue transplant products" or "cell and tissue transplantations" and have to meet all of the following criteria:

1. A cell or tissue, which is *not* substantially manipulated. Table 17.3, below, provides guidance on the definition of substantial and non-substantial manipulations;
2. Cells/tissues are used for the *same* essential function in the donor and recipients (sometimes called "homologous use");
3. It is not combined with a medical device or active implantable medical device.

These criteria are elaborated in the EU and the US legislation (see for references to the legislation BSI PAS83:2012). Whether an advanced therapy meets these three criteria is important because, if so, no clinical trials and no marketing authorization (MA) prior to commercial availability are required. If the product falls into the 'non-substantial manipulation' category only public health legislation applies. An example of such a transplantation product is a hematopoietic stem cell (HSC) transplantation which is already a well-established treatment for blood disorders since the late 1960s (see Fig. 17.5).

Advanced Therapy Classification in the EU and USA

Globally, slightly different definitions for an "advanced therapy, which is a medicinal product" are being used by regulatory bodies. In the EU, such a product is called an ATMP, which is defined as being a Somatic Cell Therapy Medicinal Product (SCTMP), a Tissue Engineered Product (TEP), a Gene Therapy Medicinal Product (GTMP) or a combined ATMP. The four subtypes of ATMPs are further defined in the following ways:

1. SCTMPs contain cells or tissues that have been manipulated to change their biological characteristics or cells or tissues not intended to be used for the same essential functions of the body. They can be used to cure, diagnose or prevent disease.
2. TEPs contain cells or tissues that have been modified so they can be used to repair, regenerate or replace human tissue.
3. GTMPs contain genes that lead to a therapeutic, prophylactic or diagnostic effect. They work by inserting recombinant genes into the body to treat cancer, genetic disorders or long-term diseases (see also Chap. 16).
4. Combined ATMPs contain one or more medical devices as an integral part of the medicine, such as cells embedded in a biodegradable matrix or scaffold (not further discussed here).

Hence, products used for HSC transplantation don't fall under the European Medicines Agency (EMA) "ATMP" definition.

See for details on these four ATMP classes Table 17.4 below.

By contrast, the US FDA's definition for ATMPs is broader than the EU's description (i.e., ATMPs + transplant products). FDA defines ATMPs as:

1. Human cells, tissues, and cellular and tissue-based products (HCT/Ps). HCT/Ps consist of human cells or tissues intended for implantation, transplantation, infusion or transfer into a human recipient and include cellular immunotherapies, cancer vaccines, and other types of both autologous and allogeneic cells for certain therapeutic indications, including HSCs and adult and embryonic stem cells (ESCs). This category covers both the transplant products and the medicinal products.
2. Human gene therapy products, which refers to medicinal products that are aimed to introduce genetic material into a person's deoxyribonucleic acid (DNA) to replace faulty or missing genetic material, thus treating a disease or abnormal medical condition.
3. Unlike the EU it is possible for human cells and tissues to be regulated as devices, however, this possibility is outside of the scope of this chapter and will not be discussed in detail (similarly to the combined ATMPs in Europe).

Hence, products used for HSC transplantation fall under the FDA "ATMP" definition.

■ What Is a Stem Cell?

The fundamental property of a stem cell is the capability to multiply i.e., it has self-renewal capacity, which is the ability to go through numerous cycles of cell division (through mitosis) while maintaining the undifferentiated state and to give rise to a variety of differentiated cells. But the general term "stem cell" is used in several contexts, each important for different reasons, as shown in Table 17.5. Adult (or somatic) stem cells, e.g., MSCs, ESCs, and induced pluripotent stem cells (iPSCs) are currently the subject of intense non-clinical and clinical investigation. Stem cells differ in the breadth of mature,

ATMP classification	Definition	Examples
Gene therapy medical product (GTMP)	A GTMP is a biological medicinal product (*excluding vaccines*) that: (a) Contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence and; (b) Its therapeutic, prophylactic or diagnostic effect relates *directly* to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence Gene therapy medicinal products shall not include *vaccines against infectious diseases* (see Chap. 14), which have their own set of vaccine specific guidances	Glybera® (see Chap. 16); Kymriah® (autologous CD19⁺ CAR-T cells)[a]; Strimvelis® (genetically modified autologous CD34⁺ cells)
Somatic cell therapy medicinal product (SCTMP)	A SCTMP is a biological medicinal product which fulfils the following two characteristics: (a) Contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor (b) Is presented as having properties for or is used in or administered to human beings with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues	Alofisel® (allogeneic MSCs); irradiated plasmacytoid dendritic cell line (allogeneic) loaded with peptides from tumor antigens
Tissue engineered product (TEP)	A TEP is a biological medicinal product that meets the following two characteristics: (a) Contains or consists of engineered cells or tissues, and (b) Is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue A TEP may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, bio-molecules, biomaterials, chemical substances, scaffolds or matrices. Products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or metabolic action, are excluded from this definition. Cells or tissues shall be considered "engineered" if they fulfill at least one of the following conditions: (a) The cells or tissues have been subject to substantial manipulation, so that biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement are achieved (b) The cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor	Spherox® (autologous chondrocytes); Holoclar® (autologous corneal epithelial cells, which contain stem cells)
Combined ATMP	A combined ATMP fulfills the following conditions: (a) It must incorporate, as an integral part of the product, one or more medical devices or one or more active implantable devices, and (b) Its cellular or tissue part must contain viable cells or tissues, or (c) Its cellular or tissue part containing non-viable cells or tissues must be liable to act upon the human body with action that can be considered primary to that of the devices referred to	Allogenic adipose derived regenerative cells encapsulated in hyaluronic acid (TEP + device)[b]; encapsulated allogeneic cells secreting GM-CSF[c] + irradiated autologous tumor cells (GTMP + device)

[a]*CD19⁺* (*CAR-T cells*) = cluster of differentiation (CD) 19 'chimeric antigen receptor T cells', CAR-T cells
[b]Hassan et al. (2013)
[c]*GM-CSF* = Granulocyte-macrophage colony-stimulating factor

Table 17.4 ■ EU-ATMP classification definitions according to the EU pharmaceutical legislation, adapted from Smith et al. (2015)

Type of stem cell	Origin	Characteristic potential (see also Table 17.6)	Application
Adult (= somatic) stem cells	Exist in small number in many tissues, often in a well-defined and supportive niche	Multipotent: Give rise to cells of the relevant tissue or local environment	Neural stem cells & limbal stem cells in pre-clinical and clinical development
MSCs (a group of adult stem cells)	A collective term for cells from mesodermal lineage, sourced from stromal or connective tissue (e.g., bone marrow, adipose tissue, and umbilical cord tissue)	Multipotent: A heterogeneous pool of cells. They have a "stem cell-like" character and can differentiate into cells of connective tissues, e.g., chondrocytes, osteoblasts, and adipocytes, but they have also been reported to give rise to many other unrelated cell types	Pre-clinical development & clinical PI-III trials; commercial (Prochymal® and Alofisel®)
Cord blood-derived MSCs (primitive stem cells, somewhere between ESCs and mature adult stem cells)	A specific source of MSCs. Extracted at birth from umbilical cord blood	Multipotent: Yet to be fully determined. Potentially they could be a source of many cell types for individual patients	Private cell banks are established for cryopreservation of cord blood samples; pre-clinical development and clinical phase I/II trials
ESC (no adult stem cells)	Result from *ex-vivo* culture of the inner cell mass of a blastocyst (embryoblast = 5–9 days old embryo)	Pluripotent	Vital source of differentiated cells for different research applications and clinical first in human (FIH) trials ongoing
iPSC (no adult stem cells	Derived by reprogramming of somatic cells (often skin fibroblasts) taken from an adult biopsy	Pluripotent, although methods for full reprogramming are still in development	From autologous source for disease modelling, drug screening including toxicity testing, and FIH trial; pre-clinical development and plans for human leukocyte antigens (HLA)-matched allogeneic iPSCs for FIH trial; research is ongoing with allogeneic iPSCs eliminating HLA-class I expression using genome editing technologies to generate universal cell lines

Table 17.5 ■ Origin, characteristics, and uses of "stem" cells

non-stem cell phenotypes to which they can give rise. These non-stem cells cannot differentiate anymore and are not capable of self-renewal (e.g., DCs, pre-ß-cells). However, some stem cells, such as skin and muscle stem cells, do only multiply and do not differentiate (i.e., unipotent stem cells). Stem cells can be characterized by their potency as defined in Table 17.6.

Before the practical applications of stem cells can be fully realized, in both the research laboratory and clinic, it will be necessary to understand in detail how to control stem cell differentiation towards mature post-mitotic phenotypes.

ESCs and iPSCs are capable of unlimited *ex-vivo* (in culture) growth. In contrast, MCSs and oligo- and unipotent stem cells cannot be grown in culture indefinitely, i.e., they grow to senescence.

■ Advanced Therapy Possible Mode of Action(s)

The *in-vivo* mode of action(s) (MoA(s)) of an advanced therapy depends on the type of cell/tissue, the *ex-vivo* manipulations performed on the cells/tissue in the manufacturing facility (e.g., genetic modification), the route of administration, and the *in-vivo* environment the cells/tissue occur. Fig. 17.2 provides an overview of the possible MoAs:

1. Pharmacological: cells/tissue release molecules such as cytokines and growth factors upon interaction with their/its environment. An example is the immunoregulatory effect of MSCs. E.g., Alofisel contains expanded adipose derived MSCs which, once activated, impair proliferation of lymphocytes and reduce the release of pro-inflammatory cytokines at inflammation sites in patients with luminal

Stem cell potency	Explanation and examples
Totipotent (or omnipotent) stem cell	Can differentiate into all embryonic and extraembryonic cell types (i.e., in humans they give rise to the foetus, umbilical cord, and the placenta: morula's cells (0–5 days old embryo)
Pluripotent stem cell	Can differentiate into all three germ cell types (endoderm, mesoderm, or ectoderm lineage) but not the placenta and umbilical cord, and subsequently into all embryonic cell types: ESCs, iPSCs
Multipotent stem cell	Can differentiate into closely related cells, such as all cells in a particular organ: MSCs, other adult (=somatic) stem cells
Oligopotent stem cell	Can differentiate into a restricted closely related group, such as a hematopoietic progenitor cell that can produce a subset of blood cell types, such as B and T cells; vascular stem cell that has the capacity to become both endothelial or smooth muscle cells
Unipotent stem cells (or precursor cell)	Have the property of self-renewal but can only give rise to cells of their own lineage, such as muscle or skin stem cells. This distinguishes these cells from real stem cells as they do not differentiate into other cell phenotypes

Table 17.6 ■ Categorization of stem cells on their potency

Crohn's disease. This immunoregulatory activity reduces inflammation and may allow the tissues around the fistula tract to heal;

2. Regenerative: *ex-vivo* manipulated cells/tissue regenerate, repair or replace a diseased or damaged human tissue. E.g., human (h)ESCs are *ex-vivo* differentiated into pre-ß cells, loaded into a device, and administered under the skin to replace damaged ß-cells of a patient suffering from DM type I.
3. Immunological: cells of the immune system are *ex-vivo* activated. E.g., cytotoxic T lymphocytes (CTL) or genetically modified (e.g., CAR-T cells) cells activate the patient's own immune system upon administration, e.g., to treat cancer.

TECHNOLOGIES

Although advanced therapies can be classified by the regulatory regime to be applied (see above), the diversity of this new group of biopharmaceuticals may be better illustrated by the underlying technology and the therapeutic use (Mount et al. 2015). Below, these technologies are briefly discussed with examples of products currently in clinical development or approved for commercial use.

■ Somatic Cell Technologies

This technology involves adult (somatic) stem cells which are isolated from a donor and subsequently *ex-vivo* manipulated using purification, propagation and/or differentiation steps to manufacture a specific cell- or tissue-based product. Thereafter, the product is administered to a patient for a therapeutic indication.

Examples of such cells are chondrocytes, HSCs, MSCs, skin stem cells, and immune cells (see Table 17.5 above). The challenge is that in each tissue a very small number of stem/progenitor cells reside and, once removed from the body, these cells grow to senescence; hence obtaining large quantities of stem cells is difficult. Also the separation of stem cells from other (unwanted) cell populations may be difficult. For some products master and working cell bank (MCB and WCB) strategies are applied, but potential changes to the genetic information and the phenotypic stability (i.e., markers present on the cell surface as measured by flow cytometry techniques) of the cells during culture over time have to be carefully assessed.

What Are Adult Stem Cells and Where Are They Found in the Body?

Adult stem cells are known to be present in many if not all individual organs in adults and are generally thought to be "multipotent," meaning they can give rise to the cells found in their organ of origin, but not in other organs (Fig. 17.3). The identification of adult stem cells in human tissues has necessitated a repositioning of basic tenets of some biological sciences, most notably in neuroscience, where the prevailing view was that no new neurons were born in humans after birth (Zhao et al. 2008). Adult stem cells are rare and they cannot always be isolated and grown in culture. Even when they can be grown in culture, usually they grow to senescence. In tissues, they exist in a defined, organized environment of supporting cells that define the architecture of the "stem cell niche" (Scadden 2006). For example, in the bone marrow there are many hematopoietic stem cell niches, each of which contain MSCs to support the function of HSCs. Each HSC is capable of producing the progenitors of all types of blood cells (Taichman 2005). Differentiation of HSCs has been studied extensively and is now well understood (Fig. 17.4), but at present conditions that allow HSCs to be maintained and expanded *ex-vivo* have not been established. A hallmark of adult stem cells is their ability to "self-renew" both *in-vivo* and *ex-vivo* and they undergo asymmetric cell division. This means that when they divide they usually give rise to two different cells, one an identical stem cell and the other a partly differentiated progenitor cell (Fig. 17.4), a process that occurs in a polarized manner controlled by the niche. The common pattern in adult tissues is that the resulting progenitor cells, sometimes referred to as "transit amplifying" cells, are capable of expansion by symmetric division and can subsequently differentiate to form the various cell types needed for repair or replenishment of the relevant tissue. Such mechanisms

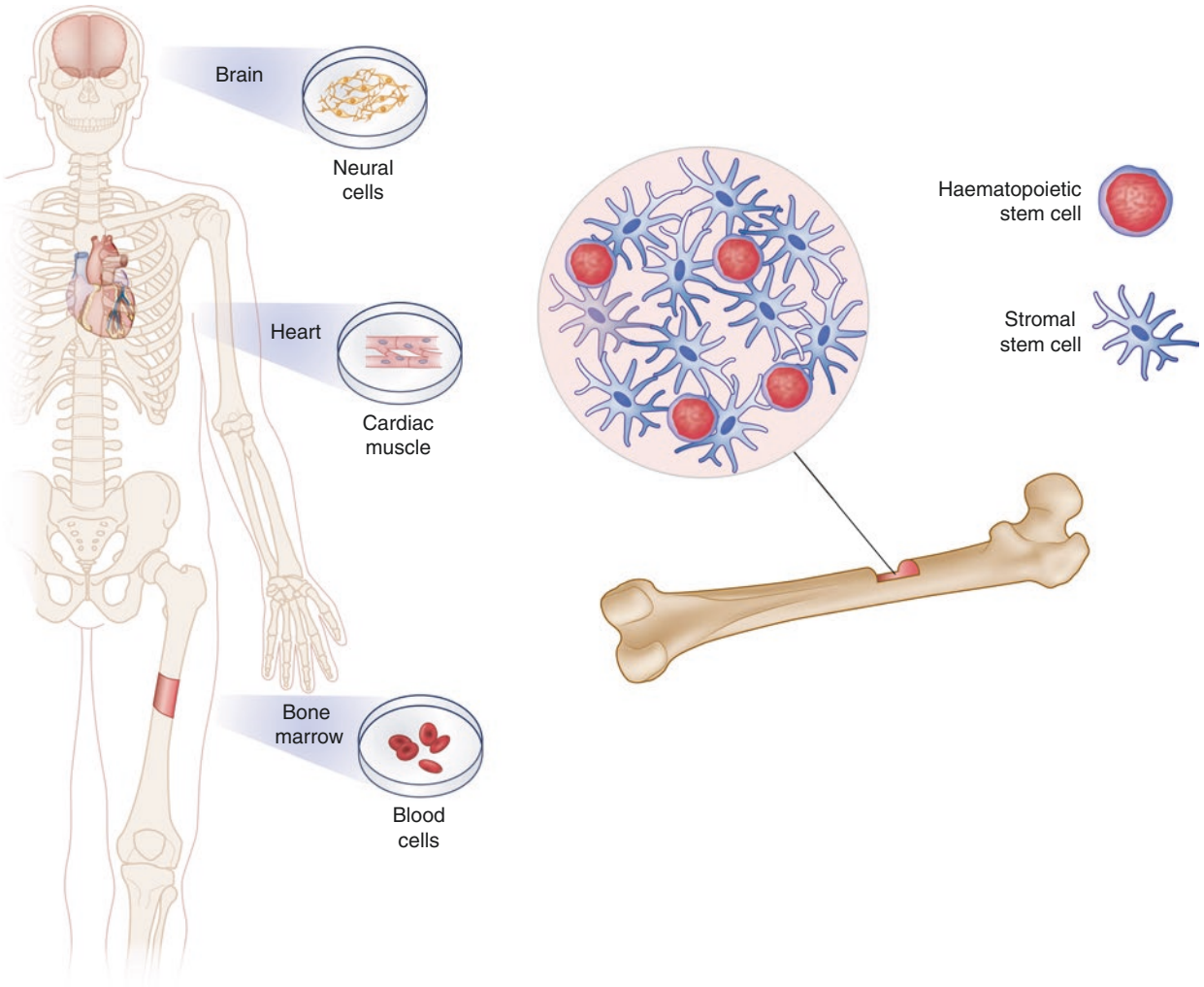

Figure 17.3 ■ Adult stem cells are present in many tissues in specific stem cell niches, giving rise to a specific group of cells found in the relevant tissue. The examples shown have been studied in detail but adult stem cells, yet to be defined, may be present in many other tissues

are well documented in tissues that are regenerated continuously in the adult, such as the epithelia of the skin, intestine and other mucosal tissues, and the bone marrow (Lander et al. 2012). Similar processes are also found in organs that are not continuously replenished, such as the brain. The realization that adult stem cells are present in many organs offers the possibility that repair and regeneration could be stimulated and controlled in degenerative diseases by drug therapy, but whether this will be possible remains to be seen. In the brain, neural stem cells have been identified in the subventricular zone and in the dentate gyrus (part of the hippocampus) (Alvarez-Buylla et al. 2001; Landgren and Curtis 2010).

Adult Stem Cells Used as Transplant Product

Adult stem cells have been used since the 1950s to treat cancers of blood cells, as one of the components of bone marrow transplants (Santos 1983). This procedure involves whole body irradiation to kill malignant cells in multiple myelomas and leukemia. The patient then receives a bone marrow transplant, not in itself a stem cell product, but the transplant contains a few HSCs which subsequently home to the bone marrow stem cell niches and begin to replenish the blood (Fig. 17.5). Rejection and graft-versus-host disease (GvHD) are still threatening complications of this form of therapy, but its practice can now be considered to be routine. These products are not medicinal products, but transplant products and fall under a different legislative regime worldwide, as discussed above.

Adult Stem Cells for Clinical Application: Immune Cells

Immune cell types currently investigated for their therapeutic value, mostly in the field of cancer, are DCs (see also Chap. 14), tumor infiltrating lymphocytes (TILs), γδ T cells, regulatory T cells (Tregs), macrophages, and viral reconstitution T cells. Both autologous and allogeneic cells are used as cell source. These immune cells have a highly specific mode of

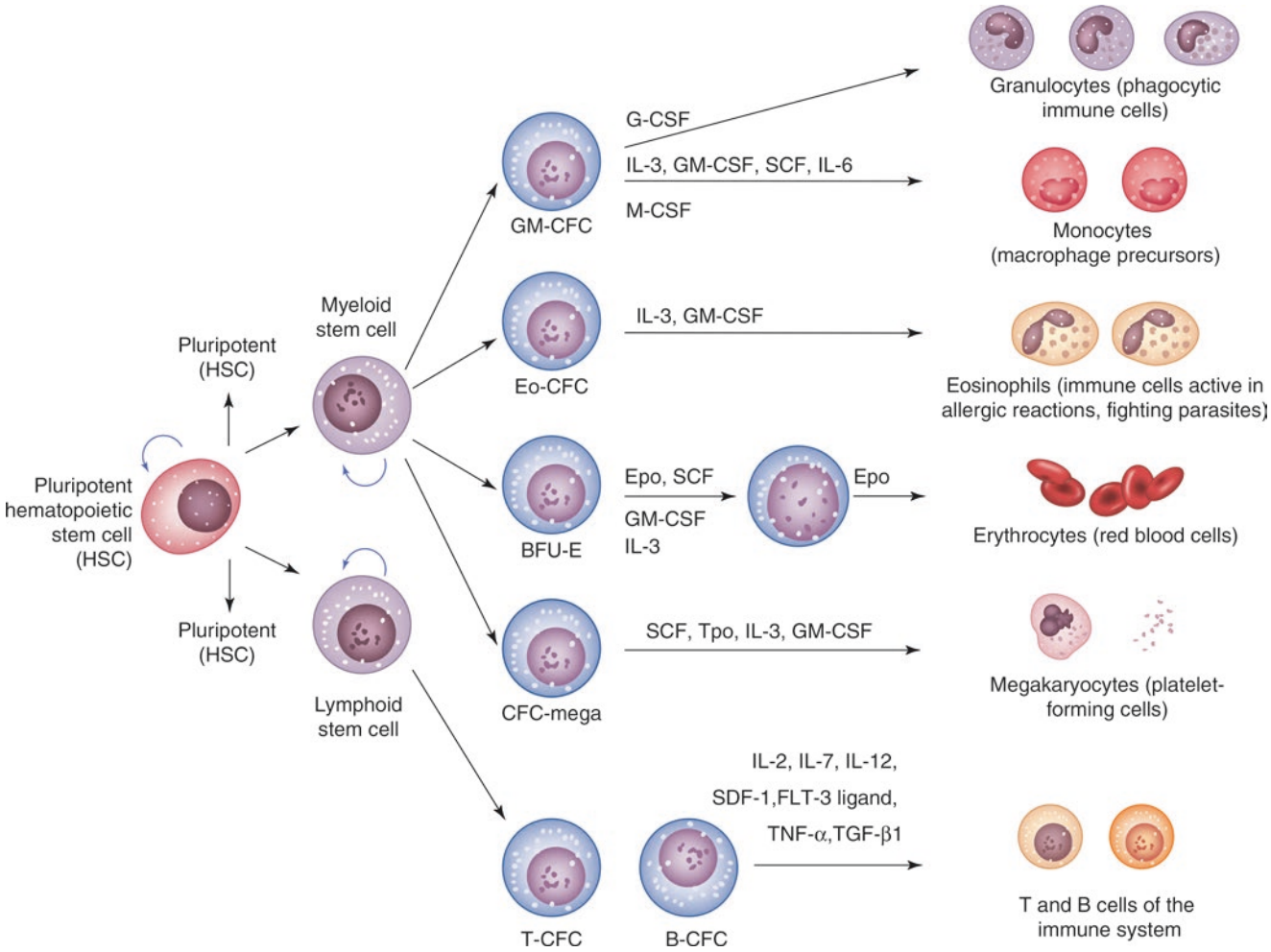

Figure 17.4 ■ Asymmetric division of adult HSCs, to produce myeloid or lymphoid stem cells, further differentiation to form mitotic progenitors, and subsequently under the control of specific growth factors and cytokines, to form fully differentiated blood cells. The differentiation pathways of the hematopoietic system are better characterized than those of other tissues, but the pattern of differentiation is typical of other tissues. *GM-CSF* = Granulocyte-macrophage colony-stimulating factor, *Eo-CFC* = Eosinophil-leukocyte Colony Forming Cell, *BFU-E* = Bone marrow erythroid progenitor cells, *IL* = interleukin, *SCF* = stem cell factor, *SDF* = stromal cell-derived factor, *TNF* = tumor necrosis factor, *TGF* = transforming growth factor

action and are in different stages of clinical development. T cells which are genetically modified using a viral vector, e.g., CAR-T cells, are much more complex to manufacture due to the modification step and fall within a different technology class, i.e., *ex-vivo* gene modification of cells using viral vector technologies (see below).

Adult Stem Cells for Clinical Application: MSCs

MSCs, sometimes called multipotent stromal cells or mesenchymal stem cells, have generated considerable interest in recent years for cell therapy applications (Bianco et al. 2008). However, the description of the cells, their source, and manufacturing processes are quite heterogeneous. MSCs can e.g., be isolated from bone marrow, adipose tissue, and umbilical cord tissue (from the particularly rich source of Wharton's jelly and also from umbilical cord blood). Because cord blood can be sampled, frozen, and banked at birth, this source of MSCs has been identified as a potential source of cells for use in a regenerative capacity in later life. There are now several private companies that offer personal cell banking services, and public cord blood banks that supply pooled cord blood samples for clinical use. Whether cord blood banking will prove to be useful remains to be seen. However, this cell source has already been tested clinically in Phase I/II trials. MSCs have been reported to differentiate into various phenotypes (including chondrocytes, osteoblasts, and adipocytes) as well as other phenotypes. Due to their pleiotropic properties, e.g., growth factors and chemokines producing, anti-apoptotic, angiogenetic, anti-fibrotic, and neuroprotective, they have been extensively tested in pre-clinical models. Hundreds of Phase I-III clinical trials have been performed and are ongoing globally in a wide variety of indications: bone/cartilage repair, heart, lung, liver, gastrointestinal, neurological diseases, and rheuma-

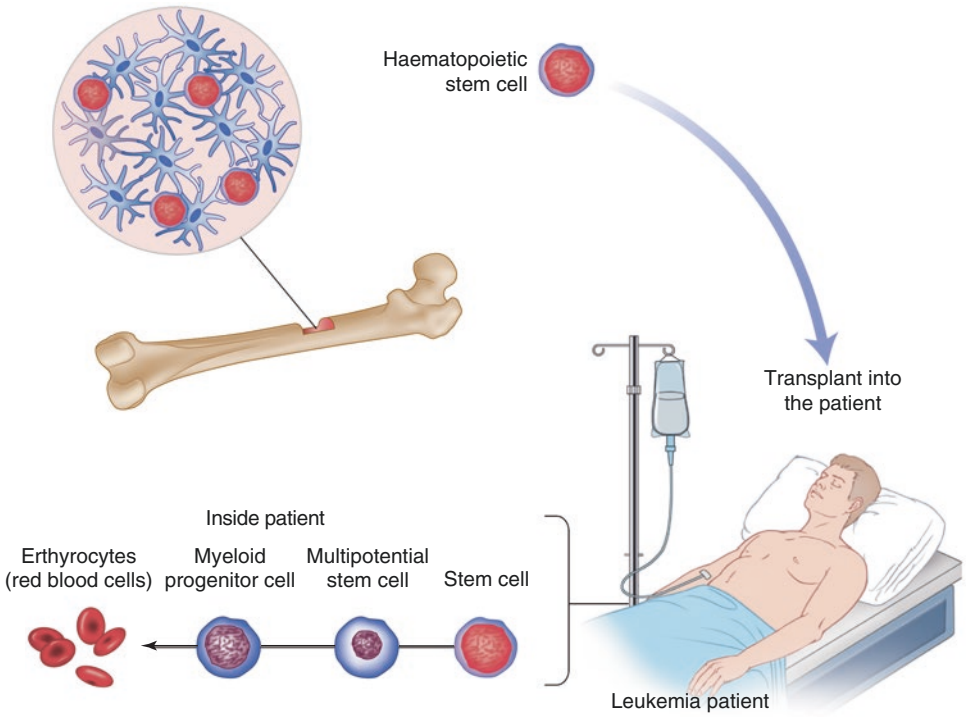

Figure 17.5 ■ Schematic representation of bone marrow transplantation, a form of stem cell therapy that was first used over 50 years ago. The transplant contains hematopoietic stem cells from the donor. These cells repopulate niches in the recipient bone marrow

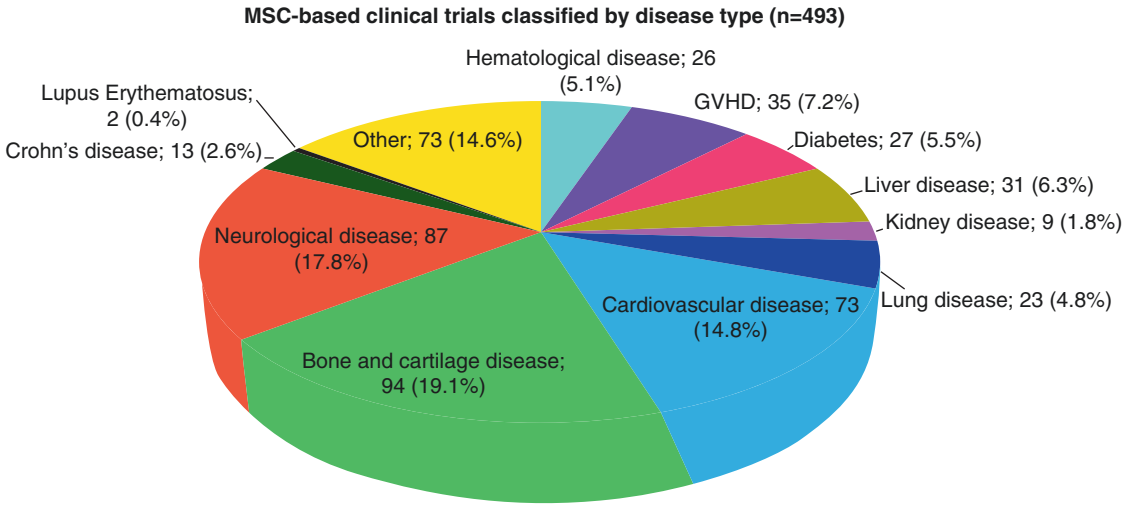

Figure 17.6 ■ Clinical trials with MSC-derived products and their indication (in percentages), adapted from Squillaro et al. (2016)

tology, Crohn's, and other autoimmune diseases, GvHD after organ transplantation, DM and kidney diseases (Trounson and McDonald 2015; Heathman et al. 2015), see Fig. 17.6.

MSC can be administered locally (e.g. intralesionally or subcutaneously) and intravascularly. While local administration has been found effective in case of local injury e.g. to treat bone and joint diseases, heart disease, for repair of muscle and ligament damage, Crohn's fistulas and even for repair of ischemic brain tissue, systemic infusion is preferable in the case of systemic diseases such as GvHD (Kean et al. 2013). Both autologous and allogeneic cell sources have been studied. To date only three MSC products have been approved globally: Alofisel in the EU for the treatment of Crohn's fistulas and Prochymal in Canada and New Zealand and Temcell HS in Japan for the treatment of pediatric acute GvHD. This is due to amongst others the lack of understanding of the therapeutic MoA, limited evidence for the preferred route of administration,

lack of delivery systems and analytical tools to characterize the cells (especially their potency), as well as limited experience on optimal manufacturing technologies to support late phase clinical trial and commercial production.

Other adult stem cell sources tested in the clinic, less frequently though, are limbal stem cells, neural stem/progenitor cells, and endothelial stem/progenitor cells (Trounson and McDonald 2015).

Cell Immortalization Technologies

Another technology makes use of immortalized cell lines as starting material for the manufacture of cell-based products. An example of such a cell line is the neural stem cell line CTX0E03, derived from human fetal cortical brain and genetically modified with a retroviral vector encoding the immortalizing gene, c-mycER^{TAM} (Pollock et al. 2006; Stevanato et al. 2009). This gene enables, under the conditional regulation by 4-hydroxytamoxifen (4-OHT), the large-scale production of the CTX cells using a two-tier cell banking system (MCB and WCB). The CTX cell-based product is currently in a clinical Phase II program for stroke. Although cell immortalization technologies have been in development for quite some time now, this is not a mainstream technology yet in the pharmaceutical world.

Ex-vivo Gene Modification of Cells Using Viral Vector Technologies

Ex-vivo genetic modifications using viral vector technology are used for several cell types; the most common are T cells, HSCs, and MSCs. The viral vector systems for transfer of genetic information into the cells are e.g., adeno associated virus (AAV), herpes virus (HPV), adenovirus (Ad), lentivirus (LV), and gamma-retrovirus (γ-RV). Cells are of autologous or allogeneic origin, *ex-vivo* purified, activated, modified with a viral vector, expanded, formulated, and filled in a primary container for direct infusion or stored frozen prior to administration. More details of the manufacturing process are described in the manufacturing and testing section below.

Gene modifications of HSCs show promise to treat diseases such as adenosine deaminase severe combined immunodeficiency disease (ADA SCID) and genetically modified MSCs are entering the FIH trials for indications such as advanced adenocarcinoma. In the case of T cells, which are currently the dominating cell type in this technology area, the approach is to genetically modify the isolated T cells in various ways to target and activate them to selectively destruct different types of systemic and solid malignancies.

HSCs Ex-vivo Genetically Modified

Since the 1990s, genetically modified HSCs have been viewed as a promising cell type for gene therapy for several reasons. Not only are HSCs readily accessible and easily separated from the bone marrow or peripheral blood; they also have the potential to expand into differentiated, long-living cell types that can carry a therapeutic gene to different sites of the patient's body, which are accessible to blood cells. Diseases of the blood and immune system, where one gene (monogenic) is involved, are now fairly well characterized and good candidates for treatment with genetically modified HSCs, as shown in Table 17.7.

Disease category	Examples
Primary immune deficiencies	ADA-SCID, Wiskott-Aldrich syndrome, granulomatous disease, X-linked syndrome
Hemoglobulin pathologies and red blood cell disorders	Sickle cell disease, thalassemia
Lysosomal storage diseases and metabolic disorders	Gaucher, mucopolysaccharidosis

Table 17.7 Candidates for *ex-vivo* HSC therapy, adapted from Scott and DeFrancesco (2016)

One of the most successful genetically modified HSC products is Strimvelis against ADA-SCID. This is the second gene therapy approved in the EU after Glybera (withdrawn shortly after approval) and it represents a historic first for *ex-vivo* gene therapy. Children with ADA-SCID are vulnerable to life-threatening infections, a result of defects in the housekeeping enzyme adenosine deaminase (ADA), which causes metabolites of adenosine to accumulate to toxic levels. To manufacture Strimvelis, patient cells are collected from the bone marrow. Then, CD34$^+$ cells (i.e., HSCs that can make lymphocytes) are extracted from the bone marrow cells. A correct copy of the gene for ADA is inserted into CD34$^+$ cells using a γ-RV vector, which has been altered genetically so that it can transfer a correct copy of the ADA gene. Once given back to the patient via intravenous infusion, Strimvelis is transported in the blood circulation to the bone marrow, where the genetically modified CD34$^+$ cells start to grow and produce healthy B- and T-lymphocytes that can produce ADA. These lymphocytes improve the patient's ability to fight infections, and so overcome the symptoms of the condition related to the immune deficiency. The effects are expected to last for the patient's lifetime, but this still has to be proven.

T-cells Ex-vivo Genetically Modified

The immune system comprises the innate and adaptive immune systems (see Chap. 14 and handbooks on immunology for explanation). Through immune surveillance, any molecules that are identified as non-self are eliminated. Targets include not only virally infected cells, as discussed in Chap. 14, but also transformed (tumor) cells,

which can become immunogenic through the expression of neo-antigens that can be recognized as non-self and can interact with an immunologically specific antibody or T cell receptor (Sharpe and Mount 2015). However, cancer cells have developed strategies to escape the immune system, which results in a failure to initiate and maintain adequate anti-tumor immunity, and consequently facilitates tumor survival and progression.

Over the last 20 years, genetically modified T cell immunotherapies have been widely tested in clinical trials, mostly in the oncology field. These cells play a key role in cell-mediated immunity. The genetic modification of the T cells occurs either through altering the specificity of the T-cell receptor (TCR) or through introducing antibody-like recognition in CARs. Both approaches enhance tumor specificities.

The potential of these T cell therapies has been demonstrated, particularly in the treatment of B cell hematological malignancies, for the following reasons: (1) B cell malignancies are relatively common; (2) B cells express several conserved cell surface markers; (3) the i.v. route provides an easy route of administration to interact with circulating B cell tumors. The product cells don't have to traffic to the tumor-site, which would be the case for a solid tumor (Fesnak et al. 2016). Most malignant B cells as well as healthy B cells express the extracellular glycoprotein CD19 antigen on their cell surface. As there is extremely limited non-B cell expression of CD19, it is an attractive therapeutic target for cell therapy (anti-CD19 CAR-Ts), see Fig. 17.7. The greatest clinical success in terms of response rates to CAR-T19 cells has been reported for patients with B cell acute lymphoblastic leukaemia (B-ALL), with one product being approved by the FDA for commercial use (Kymriah®). Yescarta® is a CAR-T19 immunotherapy commercially available in the US for the treatment of diffuse large B-cell lymphoma (DLBCL). Other cell surface markers used as a target (antigen) are e.g., CD20, CD22, and CD30. Currently, both CAR-T and engineered TCR cell therapies are also under investigation for the treatment of solid tumors, e.g., neuroblastoma, prostate cancer, breast cancer, and glioblastoma. Although successful in treatment of hematologic malignancies, the effectiveness of genetically modified T cells in the treatment of solid tumors remains modest. Table 17.8 provides an overview of genetically modified T cell clinical trials.

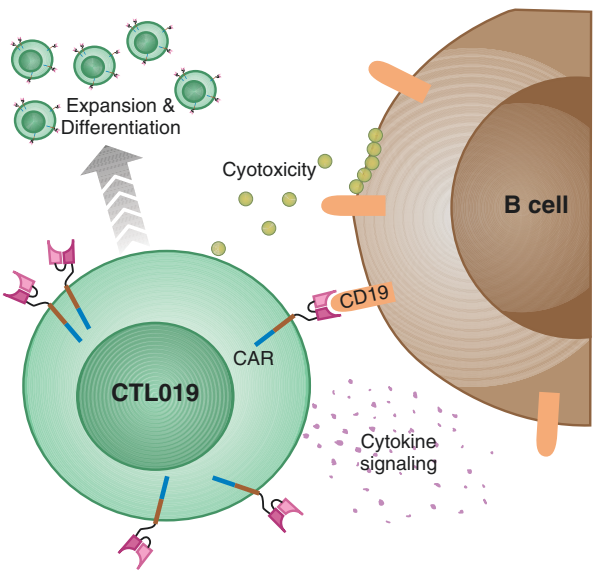

Figure 17.7 ■ Schematic presentation of Kymriah's MoA, adapted from https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM566166.pdf

Factors That Can Affect Efficacy

A number of factors may contribute to the variability observed in (long term) efficacy of genetically modified T cell therapies (Sharpe and Mount 2015). These include e.g.:

- Persistence and survival of the engineered T cells in the body.
- Patients who have shown disappearance of all signs of cancer in response to the engineered T cell treatment (complete responders), have shown greater cell persistence and survival. This may be impacted by a preparative conditioning regime (e.g., fludarabine) to reduce the number of circulating patient T cells (lymphodepletion) prior to administration of the genetically modified T cells.
- Cell dose.
- T cell therapies are normally dosed on the basis of a defined number of cells per kilogram of body weight. However, because T cells will replicate and expand after administration, which may vary among patients, there may not be a direct correlation between cell dose and efficacy. It is suggested that the ability of cells to proliferate and persist may be more important than the initial cell dose.
- Cancer cells may down-regulate or lose expression of the targeted (e.g., CD19) antigens. This may impact long-term maintenance of efficacy.
- Many factors present in the tumor microenvironment, which is composed of tumor cells, vasculature, and immune cells.
- Tumors propagate conditions that favor immune tolerance and this might impact the effectiveness of the genetically modified T cell therapy.

Factors That Can Affect Safety

A number of factors may affect safety of genetically modified T-cell therapies (Sharpe and Mount 2015). These include:

- Cytokine-release syndrome.
- Genetically modified T cells can be very effective against target tumor cells by inducing tumor cell lysis and potential tumor cell removal at a fast rate. This can result in high levels of cytokine (e.g., interferon-gamma and interleukin-6 (IL-6)) release and macrophage activation syndrome, leading to

Genetically modified T-cell therapy	Target (antigen on the cell surface of cancer cell)	Indication
Hematological tumor		
CAR-T cell	CD19 or CD20	(ALL)
		Chronic lymphocytic leukemia (CLL)
		DLBCL
		Multiple Myeloma
	CD22	B cell malignancy
	CD30	Lymphoma
	CD138	Multiple myeloma
	CD33	Myeloid malignancies
	Receptor tyrosine kinase-like orphan receptor (ROR1)	Leukaemia
Engineered TCR	Cancer-testis antigen NY-ESO-1 with/ without additional antigens	Multiple myeloma
Solid tumor		
CAR-T cell	Epidermal growth factor receptor variant III (EGFRvIII)	Glioblastoma
	Mesothelin	Mesothelioma, pancreatic cancer, ovarian cancer
	Prostate-specific membrane antigen (PSMA)	Prostate cancer
	Hepatocyte growth factor receptor, a protein tyrosine kinase (c-met)	Breast cancer
	Lewis-Y	Solid tumors and myeloid malignancies
Engineered TCR	Cancer-testis antigen NY-ESO-1 with/ without additional antigens	Various solid tumors
	Wilms tumor protein 1 (WT1)	Myeloid malignancy; mesothelioma and non-small cell lung cancer (NSCL)

Table 17.8 ■ Overview of CAR-T and engineered TCR cell-based clinical trials, adapted from Fesnak et al. (2016), Sharpe and Mount (2015)

high fevers, rigors, nausea, and diarrhea. Anti-IL-6 receptor antibody administration can inhibit these side effects.

– On-target off-tumor activity.
  This phenomenon occurs when the antigen target of the T cell therapy is expressed on normal cells as well, even at low levels. An example of this side effect is depletion of normal B cells, which also express CD19. Since B cells play an important role in the humoral immunity component of the adaptive immune system by secreting antibodies (see Chap. 14), a lack of these cells may cause infectious diseases. This side effect can be managed by the administration of antibodies. Strategies are being explored to engineer T-cells with higher selectivity for tumor than for normal cells. E.g., dual-CAR-T cells, in which T cells are genetically modified to express simultaneously two CARs with different antigen specificities (e.g., CD19 and CD123).
– Off-target reactivity.
  This side effect can occur as cross-reactivity and is particularly a risk for TCR T cells, which could react against peptides and proteins other than the target ones.

■ *In-vivo* Gene Modification of Cells Using Viral or Non-viral Vector Technologies

In-vivo gene therapy refers to the direct introduction of genetic material into the human body. This technology is discussed in Chap. 16.

■ Genome Editing Technologies

The emerge of genome editing tools, such as zinc finger nucleases (ZNFs), meganucleases, transcription activator-like effector nucleases (TALENs), and lately the cluster regularly interspaced short palindrome repeat (CRISPR)-CRISPR-associated protein 9 (Cas9) has further advanced the application of advanced therapies. Especially CRISPR-Cas9 systems are gaining interest because of signs of efficacy.

With these genetic engineering techniques, as discussed in detail in Chap. 16, it is now possible to generate human cellular disease models in a precise and predicable manner These techniques can be used to make changes in the genome which results into the formation of cells/tissues/organs identical to those of a person with a specific disease. Genome editing has been applied to introduce genetic alterations to create cardiac disease models or correct genetic mutations in e.g., iPSC- cardiac myocytes to model cardiac diseases, such as long QT- (Sayed et al. 2016; see Chap. 9), e.g., cardiomyocytes. Single genetic mutations responsible for cardiomyopathies, such as Barth syndrome and Duchenne muscular dystrophy have been corrected by use of these genome-editing tools. However, these engineered nucleases could introduce unintended genomic alterations (e.g., in addition to cleaving the on-target site, they might have off-target effects). Similarly, other challenges, such as methods for local delivery and efficiency, still need to be sorted out.

Targeted gene editing is still considered an early stage technology from a translational point of view. However, the first applications have reached the first-in-human trial phase: Human Immuno-deficiency

Virus (HIV) treatment by gene editing of autologous CD4+ T cells (CCR5 gene dysfunction) using the ZFN technology (Tebas et al. 2014).

■ Cell Plasticity Technologies

The cell plasticity technology area takes advantage of discoveries during the last 50 years that certain cells have the ability to evolve to cell types formerly considered outside their normal differentiation repertoire, i.e., hESCs and iPSCs. This technology has an extensive clinical potential due to the high probability of an almost unlimited supply of cells (MCB and WCB approach) and also for the possibility to HLA-match the resulting cell-based product (partly) with the recipient patient. The application of pluripotent stem cells, such as ESCs and iPSCs, goes beyond the administration of cell-based medicinal products and are investigated as a source for tissue engineering and organogenesis (see below three dimensional technologies). In addition, autologous and allogeneic iPSCs are currently extensively used for disease modeling (i.e., patient specific iPSC derived cardiomyocytes, cultured *in-vitro*, can be used to identify the genetic basis of a cardiac disease, leading to the identification of pharmacogenetic biomarkers that support effective and personalized drug therapy) and drug discovery including toxicity screening (Sayed et al. 2016).

Embryonic Stem Cells

During the earliest stages of mammalian development, soon after egg and sperm combine, the resulting diploid cells are said to be "totipotent," i.e., they can give rise to both the embryo and placental tissue. At the blastocyst stage of embryogenesis (day 5 in humans), the "inner cell mass" or "embryoblast" is compacted and separated from the surrounding "trophoblast." The latter combines with the maternal endometrium to form the placenta. The inner cell mass can be extracted and grown *ex-vivo* as ESCs, which can give rise to all three germ cell types (mesoderm, endoderm, and ectoderm), and therefore potentially any cell type found in the adult (Fig. 17.8). Mouse ESCs were first isolated in 1981 (Evans and Kaufman 1981; Martin 1981), but it took until 1998 for a similar procedure to be described allowing hESCs to be grown in culture (Thomson et al. 1998). ESCs can now be grown for many cell divisions, limited only by genetic damage that occurs by mutation after extensive culturing. The pluripotency of ESCs can be demonstrated in mice by injecting cells into a fertilized egg, resulting in the production of chimeric mice (i.e., mice made up of cells derived from both the donor and the injected ESCs). This process has been used routinely over the past 20 years to produce transgenic mice for research purposes. HESCs are currently investigated by a set of cell surface markers (CD markers) and their capacity to differentiate. The criteria for this assessment include the expression of surface markers and transcription factors associated with an undifferentiated state. In addition, extended proliferative capacity, pluripotency and an euploid karyotype are important characteristics of these cells. Recent evidence suggests that the epigenetic status of the cells is also a relevant criterion for hESCs. Examination of

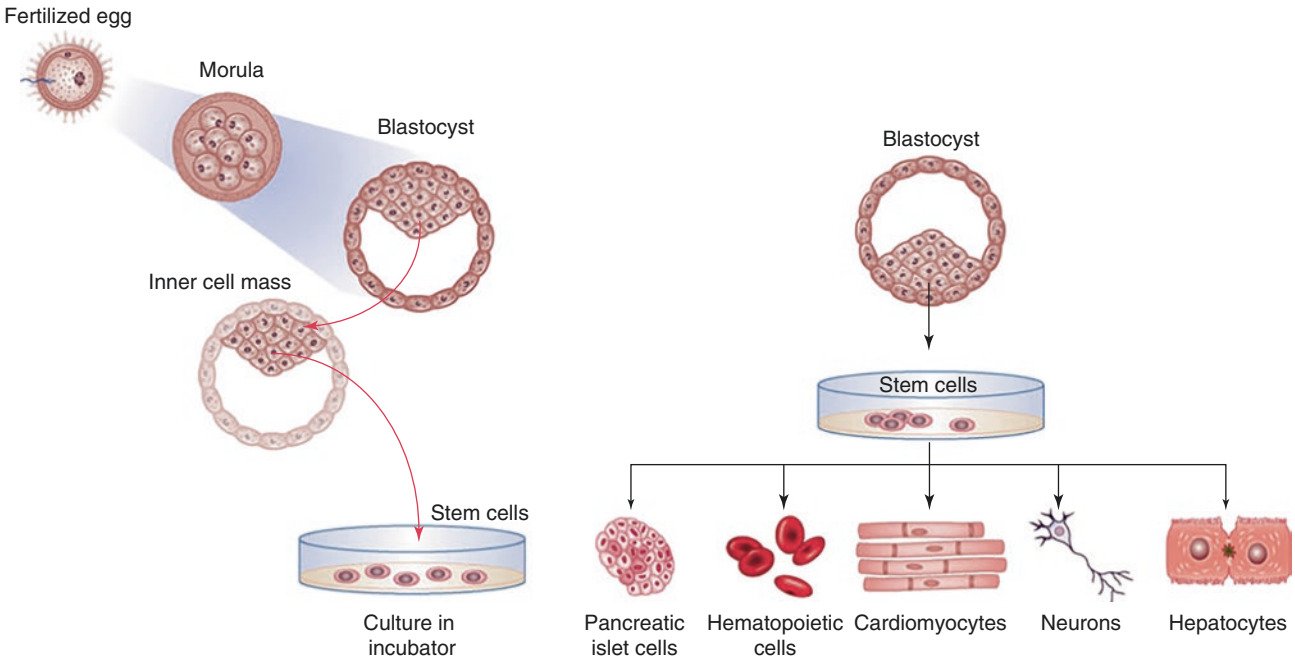

Figure 17.8 ■ Extraction of the inner cell mass of the blastocyst gives rise to ESCs, which have the capacity to differentiate into all 200+ somatic cell types found in the adult human

hESCs over extended periods *ex-vivo* should also be considered as critical parameter, to demonstrate that hESC characteristics do not change over time, and that the lines are stable in their expression of markers, expression of telomerase, ability to differentiate into the three germlines ecto- meso- and endoderm, and maintenance of a normal karyotype (i.e., number and appearance of the 46 chromosomes).

Maintenance and Differentiation of ESCs in Culture

Mouse ESCs were first grown as compact colonies on a feeder layer of mouse embryonic fibroblasts, in media containing leukemia inhibitory factor (LIF) and fetal bovine serum (FBS). Efforts to simplify culture methods soon established that the feeders could be substituted with gelatin-coated culture plates, though differentiation occurs to some extent in the absence of the feeder layer. The vital component in serum was found to be bone morphogenetic protein (BMP). Thus, mouse ESCs can be grown in chemically defined medium with LIF and BMP4 (Ying et al. 2003). HESCs are grown in the presence of high concentrations of basic fibroblast growth factor-2 (FGF2) and are unresponsive to LIF (Levenstein et al. 2006). The difference in responsiveness between mouse and human ESCs has been extensively studied and debated. The two methods of derivation may result in isolation of cells from slightly different stages of development. HESCs are thought to resemble cells from the later epiblast stage. More recently, it has been demonstrated that mouse ESCs can be maintained and grown very efficiently in the presence of small molecule inhibitors of mitogen-activated protein kinase (MEK1/2) and glycogen synthase kinase-3β (GSK-3β). This medium changes their phenotype slightly to what may be represent a "ground state" for mouse ESCs (Ying et al. 2008). A better understanding of the ground state and how this relates to hESCs will be an important step forward and will allow human ES-technology to be reproduced more effectively.

The technical challenge, now that hESCs can be maintained and expanded, is to develop robust methods to control and direct ESC differentiation, so that human cells of any desired phenotype can be obtained (Keller 2005; Murry and Keller 2008). In the context of cell based therapies, it is also important to ensure that no undesired cells are present in a product for clinical use, such as undifferentiated cells, or cells that are capable of de-differentiation (into undifferentiated cells or into cells of a different lineage), either of which could cause tumor formation after implantation, both at the site of administration or elsewhere in the body after cell migration. This science is not mature at present and will remain a priority for investigation for several years. Thus far, attention has focused on the differentiation of human ESCs towards products that could be of obvious use for clinical administration, e.g., midbrain dopaminergic neurons for Parkinson's disease, cardiomyocytes for reinforcement of damaged heart tissue, and pancreatic pre-β-islet cells for implantation in Type I DM.

At present, fine tuning of differentiation programs is still a challenge. Differentiation usually results in mixed populations of cells. For example, neural differentiation can be induced quite effectively, but the result of further differentiation is a mixed population of cells that often include both neurons and glia, and the neurons are comprised of a variety of neuronal subtypes. Timing, duration, and concentration of exposure to specific morphogenic compounds are of critical importance to the outcome and will need to be optimized in each case.

Since they were isolated for the first time, the use of hESC remains controversial. Whereas researchers clearly have experienced and experience their therapeutic potential, both pre-clinically as well as clinically, the regulators and public became widely divided, from being very supportive to seeking a regulatory ban on hESC research for ethical/religious reasons. Ocular diseases dominate these FIH trials, and these trials are showing promising safety data as well as signs of efficacy, as shown in Table 17.9.

ESC Somatic Cell Nuclear Transfer (Therapeutic Cloning)

An alternative, particularly when an HLA-donor match cannot be found, is to produce ESCs for individual patients, by somatic cell nuclear transfer (SCNT) (Wilmut et al. 2002). This process, also known as "therapeutic cloning," involves implantation of a cell nucleus from the patient (i.e., genomic DNA extracted from a skin biopsy) into a human egg, which has undergone removal of its own DNA. The environment in the enucleated egg is able to reprogram the DNA from the patient, removing epigenetic marks and restoring the DNA to an embryonic state. The development of an inner cell mass in the egg, after a period of incubation, allows extraction of ESCs that have the patient's exact genotype. These cells could be used subsequently for production of implants for cell therapy (Fig. 17.9). SCNT is also the first step in the process by which animals are cloned by "reproductive cloning," which involves implantation of the engineered egg into a surrogate mother (Fig. 17.10) (Campbell et al. 1996). Reproductive cloning of humans is illegal but is also likely to be impractical. It is known from experience with animal cloning that SCNT is an inefficient process. Most eggs that have undergone SCNT are unable to completely reprogram the donor DNA and as a result the surrogate pregnancy is usually unproductive. Even when the pregnancy comes to term, the cloned offspring is known to carry many epigenetic marks that may compromise normal development, and the famous sheep, "Dolly," the first large animal to be cloned by way of SCNT,

Indication	Active substance	Trial sponsor (country)
AMD[a]	hESC derived RPEs[b]	Chabiotech (South Korea)
Dry AMD; myopic AMD; Stargardt's macular dystrophy	hESC derived RPEs	Ocata therapeutics (USA)
Wet AMD	hESC derived RPEs	Pfizer (UK)
Dry AMD	hESC derived RPEs	Cell cure neurosciences (Israel)
Type I DM	hESC derived pancreatic endoderm cell	Viacyte/Johnson& Johnson
Heart failure	hESC derived CD^{15+} $Isl\text{-}^{1+}$ progenitors	Assistance Publique-Hopitaux de Paris (France)
Parkinson's disease	Human parthenogenic-derived neural stem cells	International stem cell Corp. (Australia)
Spinal cord injury	hESC derived oligodendrocyte precursors	Asterias Biotherapeuticcs (USA)
Wet AMD	hESC derived RPEs	The London project to cure blindness (UK)
Wet AMD	iPSC-derived RPEs (autologous)	Riken institute (Japan)

[a]*AMD* = age related macular degeneration
[b]*RPEs* = retinal pigmented epithelial cells

Table 17.9 ■ Example of clinical trials with pluripotent stem cells (hESCs and iPSC), adapted from Trounson and McDonald 2015 and Ilic et al. (2015)

had several developmental defects (Wilmut et al. 2009). Second-generation animals, produced by mating a clone with another parent, are usually unaffected by such defects, indicating that SCNT is much less efficient than the natural process of reprogramming of DNA in a fertilized egg. Given that defects are known to occur after SCNT, the subsequent derivation of cells for clinical uses might also be prone to failure due to defects in ESC differentiation. There is insufficient data available at this stage to judge whether this will be a limitation in practice. There are significant ethical concerns that have limited the practice of SCNT. A human egg donor is required, and unless the process becomes more efficient, women who are prepared to donate eggs would need to provide several eggs to produce a single ESC line. There is concern that women could be exploited, particularly women from low economic backgrounds, and as a result SCNT is not supported by government funding at present in most countries. A restricted number of ESC lines have been produced using spare eggs from *in-vitro* fertilization programs, but the status of SCNT remains a controversial topic and is subject to legal constraints that vary from country to country. An alternative source of cells for clinical application are umbilical cord blood stem cells, which are now being banked at childbirth (i.e., biobank), at least in private practice and the first clinical trials have been initiated. Whether cord blood cells can be harnessed to produce all cell phenotypes is not clear at present (see also above "cord blood derived MSCs"). However, many of the ethical issues surrounding SCNT, and uncertainty of cord blood stem cell potency (*in-vivo* activity), may become irrelevant if the promise of iPSCs can be realized.

IPS Cell Technology

Initially, work on pluripotent stem cells (PSCs) was conducted using hESCs; however, the requirement to destroy early-stage embryos in the process of ESC derivation makes their use ethically controversial. In addition, practical considerations hinders their medical applications, because any cells or tissues generated from hESCs by definition would be allotransplants into the recipient patient (see above). The discovery by Takahashi et al. (that mouse skin fibroblasts could be reprogrammed to produce pluripotent cells by forcing expression of just four genes (*Sox2, Oct4, Klf4*, and *cMyc*) using LV vectors (Takahashi and Yamanaka 2006) became a landmark in regenerative medicine. A year later similar methods were published for production of iPSCs from human fibroblasts (Takahashi et al. 2007; Yu et al. 2007). This indicated that patient-specific (i.e., autologous) pluripotent stem cells could be produced without the need for human eggs, using cells extracted from a simple skin biopsy. These cells retain similar properties as hESCs, such as indefinite growth and pluripotency. The significance of this discovery to regenerative medicine cannot be overestimated. Over the last 15 years, the iPSC field has exploded with activity, and the technology is now in use in hundreds of stem cell biology laboratories around the world. The four genes initially identified can be partly substituted by alternatives, and several experiments have shown that integrated lentiviral constructs can be avoided to reduce safety concerns, by using non-viral plasmids (Jia et al. 2010), micro ribonucleic acids (RNAs) (Yang et al. 2011), protein transduction, and even by substituting some of the factors with small molecules (Yuan et al. 2011). Often the safer alternative methods work with reduced efficiency, but nevertheless produce the same result. The technology is still in its infancy, but if it delivers its potential, iPSC technology will have profound effects on the understanding of disease by disease modeling, drug discovery and toxicity screening, correction of genetic defects, and cell-and tissue-based medici-

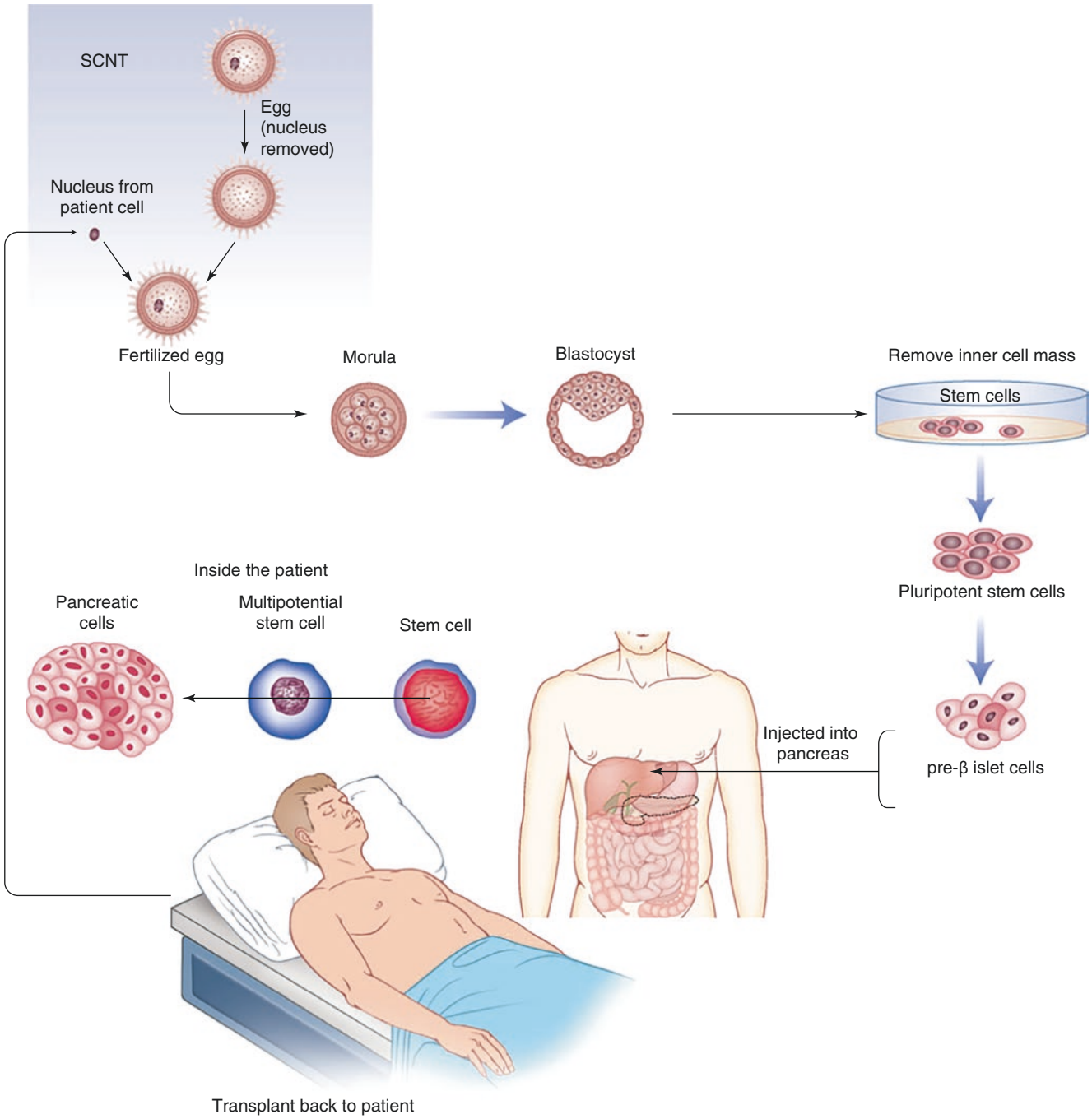

Figure 17.9 ■ Schematic diagram of the production and clinical use of cell therapies derived using somatic cell nuclear transfer (therapeutic cloning). The example given is for possible treatment of Type I insulin-dependent DM. The final maturation of the pre-β islet cells occur in the patient's body

nal products (Sayed et al. 2016). Already iPSCs have been used to correct defects in mouse models of Parkinson's disease (Hargus et al. 2010), to cure a model of sickle cell anemia in mice (Hanna et al. 2007), and other diseases (Kimbrel and Lanza 2015).

Considerable effort has been directed at investigating how iPSCs differ from ESCs and whether reprogramming is complete enough to produce truly pluripotent cells. True pluripotency is difficult to demonstrate unequivocally in human iPSCs so the development of methods to measure the extent of reprogramming will be important for practical applications. There are indications that iPSCs can have chromosomal defects and are not fully reprogrammed (Chin et al. 2010). Female human iPSCs appear to maintain the inactivated X chromosome that was present in the skin fibroblasts, though this has not been a problem with mouse iPSCs (Tchieu et al. 2010).

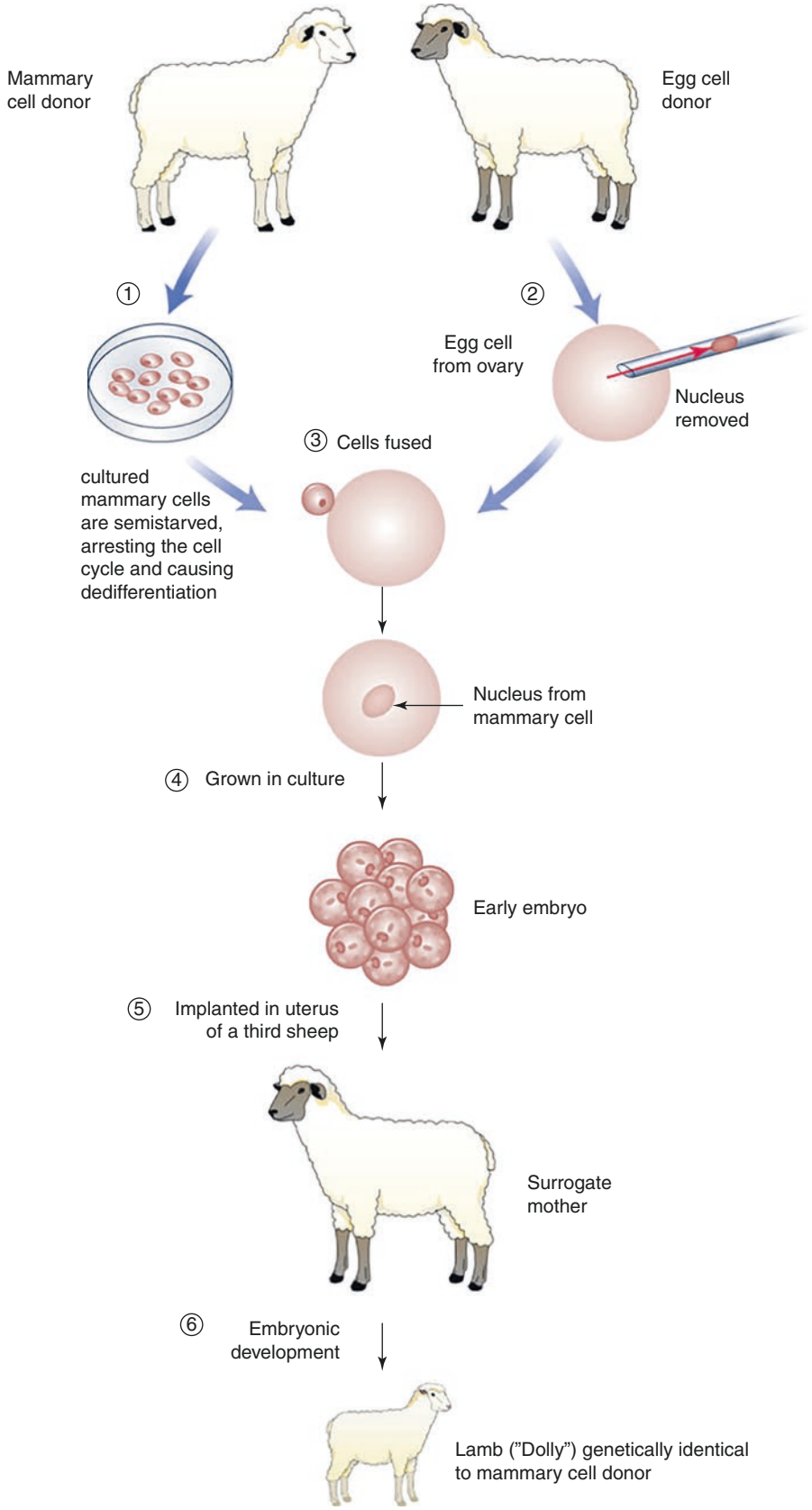

Figure 17.10 ■ Schematic diagram of the concept of reproductive cloning, as used to produce the cloned sheep “Dolly”

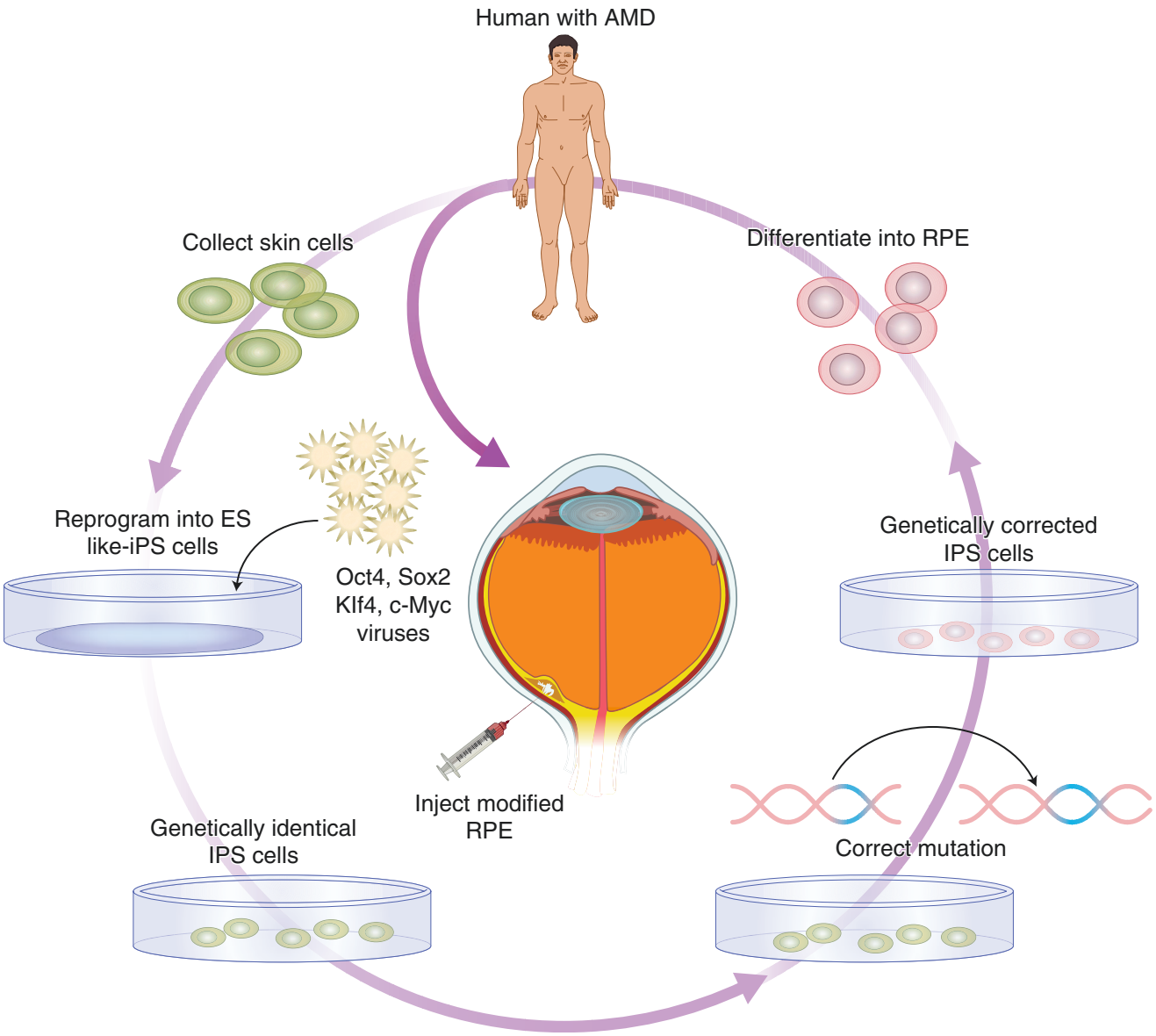

Figure 17.11 ■ Method used to produce iPS cells, correct a genetic defect responsible for AMD, and implant the corrected stem cells into humans to cure AMD

In mice iPSCs induced an immune response in a genetically identical host from which the cells were derived (Zhao et al. 2011). The mechanisms causing this immunogenicity need to be studied in more detail to investigate whether this is a widespread problem. The above unfavorable reports may be the result of inadequate control over reprogramming. In recent years, progress has been made with improved culture techniques and differentiation protocols, which resulted in safer and clinically relevant cells with lower tumorigenic risk. So far, one clinical trial has been initiated by the Riken Institute in Japan (Table 17.9; Fig. 17.11), using autologous iPSC derived RPE to treat wet AMD (Sayed et al. 2016). After the second patient was dosed, the study was temporarily put on hold due to genetic mutations found in the iPSC-RPEs (Ilic et al. 2015). The switch to allogeneic iPSC sources, both HLA-matched and HLA-inactivated, to manufacture cell based medicinal products, has the potential to broaden the accessibility of stem cell therapies to a wider population. In addition, the ability to use 'off-the-shelf' cell products makes them available for use immediately and decreases the cost of treatment compared to using the patient's own cells to generate autologous therapies, which can be time consuming and costly. Still, there is debate in the scientific community over using iPSCs derived from an allogeneic donor because of the potential immune rejection as is the case for many other allogeneic cell types. Progress has been made to address this concern through identification of HLA superdonors, individuals whose HLA profiles make

their cells widely compatible for donation to unrelated patients. Companies worldwide are generating and banking HLA superdonor iPSC lines (see above ESC lines). In Japan the first AMD patient has been treated with allogeneic skin cell derived iPSCs which were differentiated into retinal cells and transplanted onto the retina of the patient. Since the eye is an immune-privileged site, the risk for rejection of the transplant is considered low.

Direct Reprogramming/Transdifferentiation

Forced expression of genes has been used to convert fibroblasts directly into unrelated differentiated cells, including neurons (Ambasudhan et al. 2011; Wernig et al. 2008), hepatocytes (Huang et al. 2011), endothelial cells (Sayed et al. 2015) and cardiomyocytes (Burridge et al. 2012) by skipping the iPSC stage. The technique used is analogous to that used to derive iPSCs, except that genes associated with the desired somatic cell are expressed instead of pluripotency genes. The realization that cellular phenotypes can be transformed in this way has been met with astonishment and is certainly breakthrough technology. It raises the possibility that interconversion could be performed *in-vivo*, though it does not allow for expansion of cells in preparation for an implant. However, direct reprogramming of fibroblasts to neural stem cells, as reported in 2012 (Han et al. 2012; Thier et al. 2012), may be a short cut to neurons. This approach may offer some advantages over production of neurons by way of iPSCs.

■ Three-Dimensional Technologies

Another technology, tissue engineering, is combining somatic cell technologies or cell-therapy technologies described above, with various types of biocompatible materials to solve structural challenges that are often surgical or immunological in nature. Three-dimensional (3D) technologies, including biomaterial scaffolds, can have many purposes, such as supporting cell viability, induction of cell differentiation, provision of a substrate for cell growth and support of tissue regeneration, provision of the shape, scale, and volume of a desired tissue, provision of growth factors, and encapsulation of cell-based products to protect the product from the host immune system to avoid rejection. This is schematically presented in Fig. 17.12 (Smith and Grande 2015). 3D technologies can be divided into four subtypes of technologies as shown in Table 17.10. For further reading see Murphy and Atala (2014); Wegst et al. (2015); Smith and Grande (2015); Pedersen et al. (2012); Kim and Matsunaga (2017).

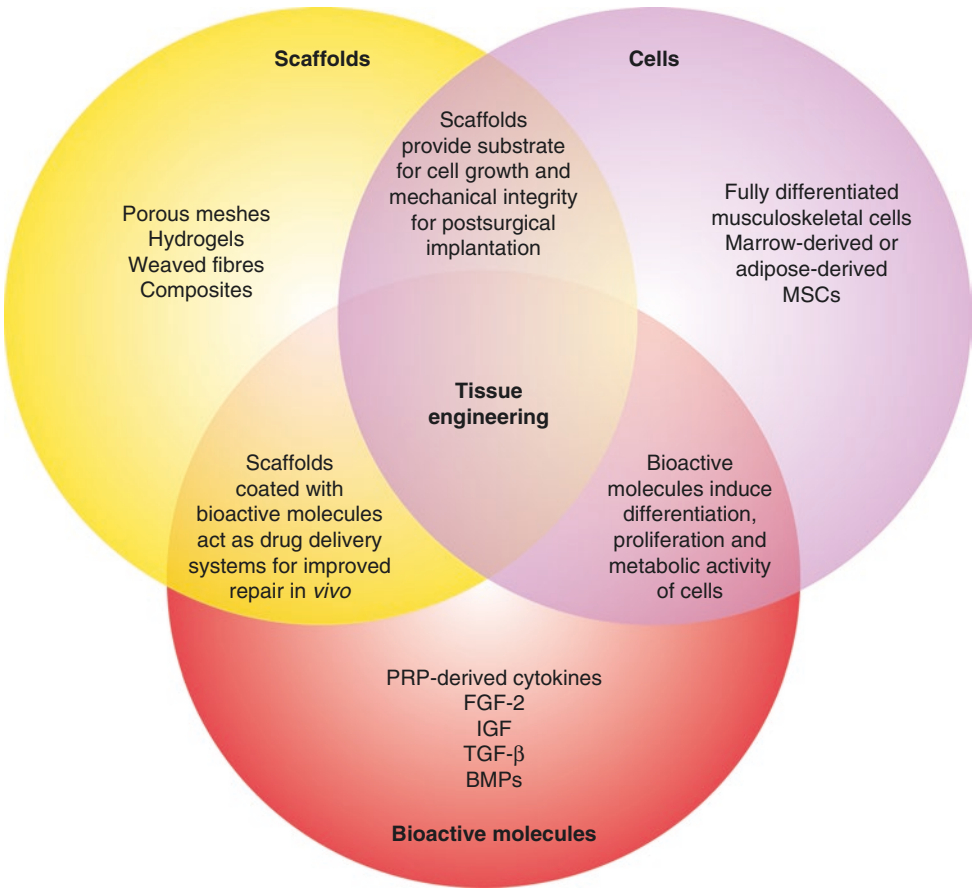

Figure 17.12 ■ The role of scaffolds in tissue engineering strategies. Scaffolds are an important component of the tissue engineering triad. *BMP* = bone morphogenetic protein, *FGF-2* = fibroblast growth factor 2, *IGF* = insulin-like growth factor, *MSC* = mesenchymal stromal cells, *PRP* = platelet-rich plasma, *TGF-β* = transforming growth factor β. adapted from Smith and Grande (2015)

Subtype of 3D technologies	Examples of products/organs in pre-clinical or clinical development or commercially used
Simple biomaterials e.g., hyaluronic acid, bone substitutes, alginate-encapsulated islets	Allogenic adipose derived regenerative cells (keratinocytes) encapsulated in hyaluronic acid to regenerate extracellular matrix–like material to treat corneal blindness; transplantation of pancreatic islets in immune protective alginate capsules to treat DM Type I; MACI® for repair of cartilage defects of the knee (see Figure 17.13)
3D/shaped scaffolds that provide organ shape and bio-resorbable substrate for cell growth	Bladder; trachea; 3D-printing technologies
Tissue-derived (decellularized) scaffolds that are 3D but with added benefits of native biomechanical strengths and matrix factors	Esophagus; trachea
Smart (second generation) biomaterials that may have thixotropic, thermo-responsive, growth-factor-encapsulating or *in-situ* self-assembly properties	Chitosan and hyaluronic acid are typically used as excipients for thermoset injectable hydrogels encapsulating cells

Table 17.10 ■ 3D technologies and examples

■ Combinations of the Above Technologies

A combination of the above technologies is currently in pre-clinical development in the cell therapy area, e.g., the self-formation of complex organ buds into organ-like structures, i.e., organoids (Sasai 2013).

NON-CLINICAL ANIMAL TESTING CONSIDERATIONS

A full pre-/non-clinical testing program during drug development as presented in Chap. 8 for mAbs, may not always be feasible or necessary for advanced therapies due to the nature of these products, consisting of a heterogeneous population of human cells or tissues (see also Table 17.2). Generally, the pre-/non-clinical testing package entails studies to provide data on the following:

(a) safety (toxicity, including immunogenicity);
(b) tolerance (local, systemic);
(c) biodistribution;
(d) persistence (duration of exposure);
(e) *in-vivo* proliferation, maturation, and/or differentiation into an unwanted lineage of stem cells (ESCs, iPSCs);
(f) tumorigenicity;
(g) reproducibility;
(h) biological activity (potency) *in-vivo* and/or *in-vitro*; *in-vivo* mechanism of action
(i) *in-vitro* and *in-vivo* efficacy studies to understand which cells/cell-sub-populations and cell characteristics have therapeutic value;
(j) PK/PD to serve dose definition, e.g., number of (viable) cells;
(k) PK/PD to serve route of administration and schedule;
(l) study duration to monitor for toxicity;
(m) safety of surgical procedure for local delivery of cells/tissues.

Non-clinical animal safety (toxicology) and efficacy (pharmacology) studies pose significant challenges when applied to advanced therapies, e.g., for the following reasons:

1. Molecular incompatibility and immune rejection in xenogeneic human-animal combinations (i.e., human tissues/cells tested in animal models). This is also true for genetically transduced cells, where the genetic modification leads to the expression of human protein(s), e.g., CAR-T cells.
2. A cellular immunotherapy to treat cancer (e.g., TILs) relies on interaction of the cellular product with the patient's immune system for its effect. The *in-vivo* immunological effect will very likely be different between species.
3. Cells do not undergo ADME in a way conventional medicinal products often do.

Without non-clinical data it may be difficult to predict the potential safety of the proposed first-in-human clinical studies. Therefore, alternatives should be investigated that could yield evidence of safety and, for late stage clinical trials, evidence of efficacy, including the use of models explained in Table 17.11 (adapted from BSI PAS83:2012).

■ Relevant Animal Models

Mice are often the species of choice to study advanced therapies. They are relatively inexpensive, reproduce quickly, and can be easily manipulated genetically. However, the ability of mouse experiments to predict the effectiveness of advanced therapies remains controversial. The failure of many mouse models to precisely predict particular human diseases has compelled investigators to examine animal species that may be more predictive of humans. Larger animals, such as rabbits, dogs, pigs, goats, sheep, and non-human primates, are potentially better models than mice for this

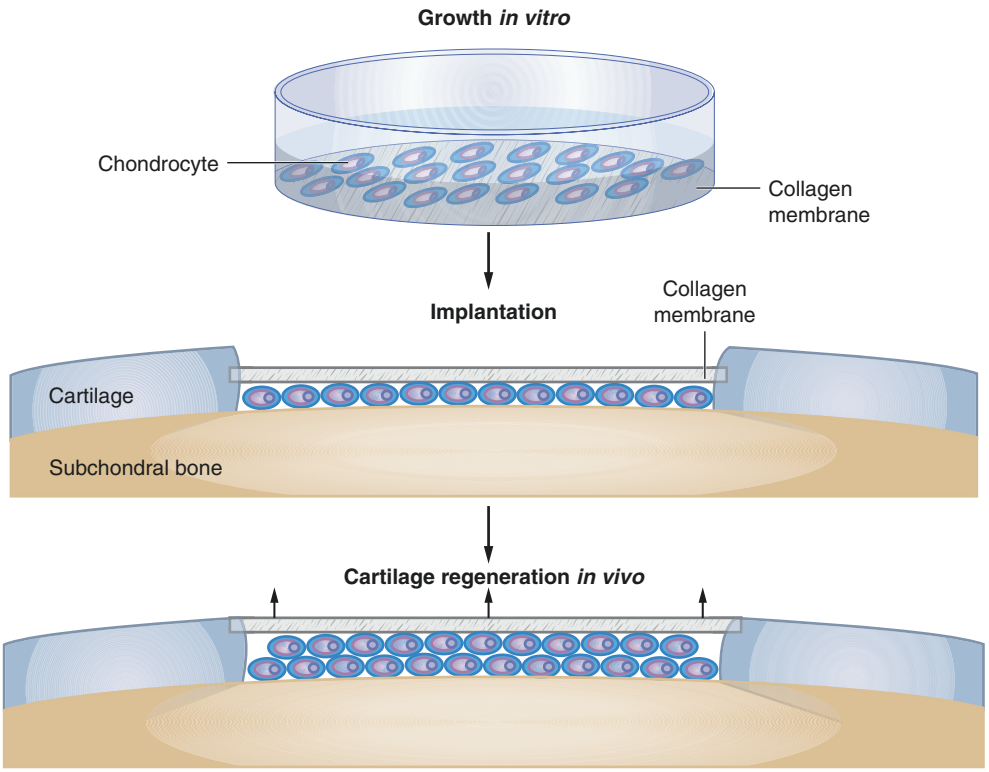

Figure 17.13 ■ Matrix-assisted chondrocyte implantation (MACI) in cartilage repair. MACI uses chondrocytes that have been seeded into a collagen scaffold and cultured for a period of time prior to surgical implantation, adapted from Smith and Grande (2015)

purpose. They have a longer life span, which facilitates long-term (e.g., years) studies that are critical for some advanced therapy products with a life-long effect. Many physiological parameters (e.g., immune system properties that play an important role in the reaction of the host animal to advanced therapies) are much closer to humans than are those of rodents. Large animals also have significant advantages regarding the number and types of cells or amounts of tissues that can be reproducibly isolated from a single donor animal and *ex-vivo* manipulated in sufficient quantity for analysis and for various non-clinical applications.

In case animal safety data do not provide meaningful information based on which an extrapolation can be made to potential risks posed to humans, those studies may be (partially) waived by regulatory authorities. Study set-up and duration for evaluation of the toxicity and/or biodistribution have to be determined on a case-by-case basis and depend on, e.g.:

- product half-life which may vary between hours–days and months–years, the latter for cells which engraft in a specific niche in the human body;
- potential alterations of cells over time upon administration;
- dose regime of single or repeat dosing over a period of weeks–months–years;
- chance for migration of the cells in the body to unwanted sites upon administration (e.g., local administration of an adult stem cell in the sub-retinal space of the eye may be safer than the systemic administration of an ESC/iPSC derived product);
- type and number of *ex-vivo* cell manipulations performed during manufacture (i.e., in case cells are expanded for multiple passages close to the point where these cells senesce, animal studies should be performed with cells beyond the cell passage used to manufacture the advanced therapy).

Generally, genotoxicity and specific safety pharmacology studies are not conducted for cell and tissue based products, unless there is a reason for concern (e.g., novel excipient or novel route of administration for an approved excipient). Reproductive toxicity studies are only required when there is a potential risk for exposure to the reproductive organs.

And finally, literature data may be used to support the (lack of) animal data. See Herberts et al. (2011) and Vestergaard et al. (2013) for further reading.

CLINICAL CONSIDERATIONS

For investigating the safety and efficacy in humans, generally the same principles apply to advanced therapies as to other medicinal products (see Table 17.2). However,

Animal and other model options	Example	Comment
Immunodeficient or immunosuppressed animal	NOD.SCID-rd1 mouse model of retinitis pigmentosa	See Chap. 9 for details on transgenic animal models
Animal disease model	Diabetic mouse model	Not always possible especially in case of immune based disease
Homologous animal model	AMD mouse model	Copy of human condition regarding pathology, symptoms and prognosis of disease. Use species specific autologous or allogeneic cells instead of human cells and apply the same manufacturing process to produce the animal cell based product; characterize the product to the extent possible; mimic the clinical setting in terms of route of administration, surgical procedure, and dose regime, to the extent possible
Homologous animal model plus use of a vector	ADA-SCID mouse model	See above plus vector encoding the animal homologue for animal cell transduction
Non-invasive whole animal modeling system	Magnetic resonance imaging (MRI) or computed tomography imaging (CTI) techniques	Cell fate/biodistribution studies in animals
Large animal model	Delivery of cells in the sub-retinal space of a pig's eye to train surgeons to safely administer cells in the eye of AMD patients; delivery of stem cells for treating spinal cord injury in pig model	Development of complex surgical procedures which would be technically difficult or impossible in small species
In-vitro assay system	Cell culture system to mimic cell migration upon immune stimulus	Potency test to characterize an advanced therapy

Table 17.11 ■ Examples of animal and other pre-clinical models applied for assessment of safety, efficacy, and product potency testing

considering the nature and complexity of the products and potential risks and benefits, there are some unique aspects to the clinical programs (see Mount et al. 2015):

1. Different set-up of trials compared to most conventional medicinal products:
   (a) First-in-human trials are always in patients and never in healthy volunteers;
   (b) A seamless development path rather than the traditional route of separate formal phase I (safety), phase II (hint of efficacy), and phase III (safety confirmation and efficacy) studies.
2. Traditional PK (ADME)/PD studies may not be feasible.
   (a) Dose (defined as number of cells/mL; number of cells/kg body weight) escalation studies may not be feasible as there may not be clear dose-response correlations. Often, a low-, medium-, and high-dose are selected, based on literature data concerning the number of cells that have historically been administered to humans.
   (b) Advanced therapies are frequently administered via the intravenous route and rapidly cleared via the lungs, spleen and liver (Leibacher and Henschler 2016). Other possible routes are intranodal (DCs to treat rheumatoid arthritis) or local administration via a surgical procedure, e.g., into the eye, brain, spinal cord or knee.
3. For safety evaluation, the following risks may need to be taken into account, depending on many factors, including type of product, cell differentiation status upon administration, cell proliferation capacity, cell source being autologous, allogeneic or xenogeneic, half-life of the cells in the body/lifelong persistence, site and method of administration/implantation, quality of the starting material (derived from healthy donor or very sick patient), and disease environment(s) which cells may encounter in the patient's body:
   (a) Tumor formation (tumorigenicity) e.g., in case of ESC- and iPSC-derived products which are *ex-vivo* expanded and differentiated;
   (b) Potential adverse reactions at the site of administration e.g., dimethyl sulfoxide (DMSO) related side effects upon i.v. administration;
   (c) Cells, being sub-visible particles, make it difficult to assess sub-visible particles potentially present in the product. These foreign particles may damage the tissue upon administration e.g., in the sub-retinal space of the eye;
   (d) Inflammatory responses and infections (e.g., side effect of CAR-T cells);
   (e) Implantation procedure for cells or 3-D tissue replacement therapies using a complex surgical

procedure (e.g., to administer cells in the subretinal space of the eye, in the spinal cord or in the brain; 3-D cultured trachea placed in the throat);

(f) Immuno-mediated side effects (CAR-T cells may cause cytokine release syndrome);

(g) Immunogenicity, which may depend on:
- Relative immune privilege of the administration site (e.g., eye);
- Allelic differences between product and patient cells (e.g., allogeneic dendritic cells);
- Immune competence of the patient;
- Need for repeat dosing (more doses may increase the chance of immune rejection of the advanced therapy);
- Maturation status of the cells (e.g., ESCs).

Often, advanced therapies derived from an allogeneic cell source require immune-suppressant medicines to be administered together with the cell-/tissue-based product. However, some allogeneic cell-/tissue-based products, such as MSCs, have shown relatively low immunogenicity profiles, in part due to the short half-life of the cells in the body. See for more details below.

4. Selecting the right patient population for the initial clinical program is challenging as there is a tension between choosing the patients most likely to benefit from an efficacious advanced therapy (e.g., early stage cancer patients) and limiting the risk to which patients are exposed with the unlicensed therapy (late stage cancer patients who may not benefit from the therapy at all due to their severe illness).
5. Establishment of surrogate biomarkers for efficacy assessment may be needed to predict long-term clinical outcome of cells that may persist in the body for years, e.g. CAR-T cells which engraft in the peripheral blood and bone marrow and transduced $CD34^+$ cells, which engraft in the bone marrow.
6. Particularly for genetically modified cells, which may persist in the body for many years or lifelong, long-term (10–20 years) patient follow-up for safety, efficacy, and durability monitoring may be necessary.

■ Immunological Considerations in Advanced Therapy

The potential application of adult stem cell based medicinal products derived from allogeneic source as well as hESC based therapies is limited by risks for graft-host rejection issues, as with all therapeutic strategies based on cell, tissue and organ transplantation, unless the transplant is derived from an autologous source. A way to overcome this challenge is the use of a device to protect the allogeneic cellular product from the host immune system. An example of this strategy is Viacyte's cell-based combination product, where the hESC derived β-islet progenitors are contained in the Encaptra® cell delivery system, which is placed subcutaneously (see Table 17.9 and Fig. 17.19). The additional advantage of this system is that cells cannot migrate in the body to unwanted sites and the device can be taken out in case of e.g., tumor formation. The disadvantages of such an immune-protective device are fibrosis and the lack of vascularisation around the device, required for cell viability and insulin production. Certain sites of the human body have immune privilege, i.e., they tolerate the introduction of non-self-antigens without eliciting an inflammatory immune response. These sites include the eyes, the testicles, the fetus and certain tumors. There is debate in the cell therapy world regarding the immunogenicity of allogeneic MSCs (Consentius et al. 2015; Ankrum et al. 2014). Clinical trials with standardized immune monitoring programs and a better understanding of the *in-vivo* mode of action of allogeneic MSCs may provide answers.

Administration of drugs to suppress the immune response is standard practice for patients undergoing transplantation, but with immunosuppression come side effects and uncertainty. The hope is that iPSC technology (see above) may overcome rejection problems but it is too early to be sure at this stage as there is only one product tested clinically in one subject and this is from an autologous cell source (Table 17.9). Another approach is to bank a collection of ESC lines that allows selection of a matched ABO and HLA haplotype or a close match (Lui et al. 2009). It has been estimated that with a bank of 70–100 ESC lines, a partially matched ESC line can be chosen that is adequate for each recipient. The downside of this approach is that at the time the cell lines are banked, it may not be clear yet for which diseases they will be used in the future, hence what the critical parameters are to characterize the banks for (e.g., purity of the cells, stability, potency, viral safety), see Bravery (2015). Preparing cell banks, extensive testing, and long term storage under frozen conditions are very expensive undertakings.

MANUFACTURING AND TESTING CONSIDERATIONS

■ Manufacturing

Cell and tissue based products are distinct from traditional biopharmaceuticals in that the modified cell/tissue itself is the active ingredient in the medicinal product rather than "simply" the means by which the cells produce an active ingredient (e.g., a recombinant protein; a viral vector). However, many of the production platforms, cell culture media, storage and transport bags, and product excipients and primary containers, which have been established for traditional cell-based recombinant protein manufacturing processes (see other chapters), can be readily applied to these innovative products.

Manipulation step	Equipment used (examples)
Collection or generation of autologous or allogeneic donor cells; collection of tissue biopsy (i.e., starting material). This step is not considered a GMP manufacturing step and takes place outside the GMP facility at a clinical site	Bone marrow aspiration system, surgical procedure, apheresis/leukopheresis system (Fig. 17.14)
Isolation of specific cell population(s). This is usually where the GMP manufacturing process starts	Knife; fluorescence-activated cell sorting (FACS) (see below); positive/negative selection by e.g., magnetic-activated cell sorting (MACS®) technology (microbeads and column); Elutra®; LOVO spinning membrane filtration device
Cultivation, expansion, and/or (genetic) modification of cells; tissue culture	Cell culture systems (see Chap. 4)
Cell differentiation	Specific raw materials, such as growth factors, are added to the culture medium manually or automatically
Purification of desired cell population(s); purification of tissue	Counter-flow centrifugal elutriation (Ficoll). This technique separates cells by size and density while maintaining cell viability. Cell enrichment kit for the magnetic separation of the desired cells by negative selection. It utilizes antibody magnetic bead complexes. Undesired cells are bound by the antibody and then magnetic beads that, when placed in a magnetic field, leave the desired cells untouched and free in the medium. The same principles and systems can be applied as for isolation of specific cell population(s) (see above)
Cell harvest and cell wash/cell concentration; tissue harvest and wash	Centrifuge; fluidized bed + elutriation-closed system (K-Sep); tangential flow filtration (TFF) technology; spinning-membrane filtration;
Formulation of the harvested cells in excipient mixture; formulation of tissue	Manually; mixing station with disposable bag set-closed system (Invetech)
Filling in the primary container of cell suspension; transfer of tissue to primary container (this is considered the drug product (DP)	Manual vial filling, stopping, and capping (Flexicon pump); manual bag filling and sealing; (semi) automated vial filling (FPC50, Flexicon system)
Labelling of the primary container	Manually; automatically with labelling machine
Short/long term storage of the DP	Refrigerator; controlled rate freezer; freezer, cryopreservation tank
Shipment of the DP to the clinical site	Temperature controlled shipment in cool box, on dry ice, in cryogenic Dewar
Handlings of the DP at the clinical site to allow for administration of the DP to the patient (e.g., thawing, washing, mixing with other ingredient)	Plasmatherm controlled temperature rate dry thawing instrument; centrifuge, mostly manual handlings

Table 17.12 ■ Typical advanced therapy manipulation steps and equipment used for each step

Since the fast majority of advanced therapies contain viable cells/tissue that can be easily destroyed through sterilization procedures and cannot be sterile filtered (≤0.2 μm filter pore size), as cells have a size of 10–30 μm on average and tissues are even bigger, the manufacturing of these products must take place under aseptic conditions. For non-sterile raw and starting materials as well as excipients, additional steps may need to be taken to ensure subsequent aseptic manufacturing (e.g., heat inactivation, gamma-irradiation or sterile filtration of the material). The facilities, equipment, raw materials, viral vectors, and cells/tissues used must be of suitable quality to allow for good manufacturing practice (GMP) production of the drug product for human application. At every stage of production, materials and final product should be protected from microbial, viral, and other contamination.

The manufacturing of advanced therapies typically requires many or all of the following "manipulation" steps, see Table 17.12.

Control of Manufacturing Process

As for any biopharmaceutical manufacturing process, process variables need to be chosen carefully and monitored to allow for adjustments to the process and to ensure a product of high quality is consistently produced. Process variables assessed are e.g., medium perfusion or exchange rate, feeding regime, biomass, stirring speed, pH, dissolved oxygen (DO), and lactate production. Particularly in the case of open and manual culture steps, this is challenging because any handling of the cells/tissue may impact the quality of the viable material and could potentially contaminate the culture system. Examples of fully-closed production

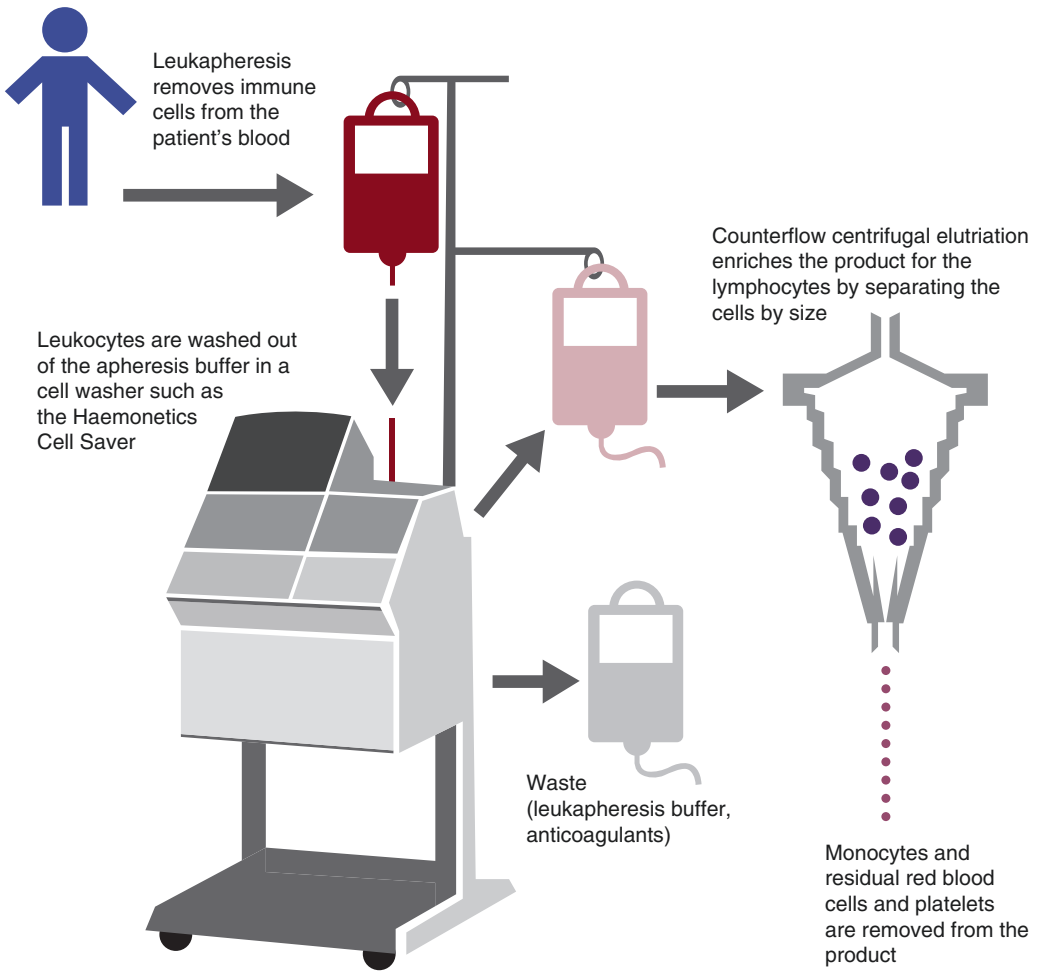

Figure 17.14 ■ Example of a leukapheresis system, which collects lymphocytes from the donor's peripheral blood, reprinted with permission (Levine et al. 2017)

systems enabling different manipulation steps in one system are the CliniMACs Prodigy® and the Octane Technology (see Figs. 17.15 and 17.16, respectively).

A fully closed processing system is the CliniMACS Prodigy. This is a single-use device that performs all manufacturing steps (i.e., cell wash, enrichment, activation, genetic modification, expansion, final formulation, and sampling). This contrasts with other manufacturing approaches, which use separate machines for the cell culture, cell washing, and other steps in the production chain.

The manufacturing process for advanced therapies show parallels with the processes for E.coli/mammalian production cells described in Chap. 4 for therapeutic proteins. But they differ considerably from those processes at a number of critical points. On top of that, the various types of cell therapy products vary widely between each other. Below follow examples of manufacturing process flow-charts for three different types of advanced therapy medicinal products:

1. *Off-the shelf* or *non-off-the-shelf* MSC production process, as described below and presented in (Fig. 17.17);
2. *Non-off-the-shelf* CAR T production process, as this procedure is a prime example of "personalized medicine" (see Chap. 9) the complexity is caught both in the text below and shown in Fig. 17.18;
3. *Off-the-shelf* human ESC derived pre-beta cell production process, as described below and presented in Fig. 17.19.

Manufacturing of MSC Product

The manufacturing of an off the-shelf (allogeneic) or non-off-the-shelf (autologous or allogeneic) cell based product, e.g., MSC-derived product, is a multi-step process with slight modifications for each specific product (see Fig. 17.17):

- Step 1: Starting material procurement via bone marrow (BM) aspiration (1a) or adipose tissue biopsy (1b) from a healthy donor (allogeneic cell source) or patient (autologous cell source). Other sources of

MSCs are not discussed here. The donor (healthy person or patient) is tested for specific human viruses prior to donation of the starting material.

- Step 2: Mononuclear cell separation from BM (2a) using separation techniques; adipose tissue digestion using enzymes, such as collagenase (2b).
- Step 3: Mononuclear cell separation from digested adipose tissue.
- Step 4: MSC expansion: MSCs are adherent cells and can therefore either be cultured in a culture flask (2D culture) or on micro-carriers in suspension culture (3D culture). Cells grow and multiply via mitosis and meiosis. By selecting the appropriate surface and culture medium, and culture conditions, unwanted cell populations do not adhere and are separated from the wanted cell populations.
- Step 5: Cell detachment from the surface via trypsinization. Cells are washed to remove dead cells, unwanted cell populations, and trypsine. Steps 4 & 5 are repeated as many times as needed for the targeted dose or to freeze-down a cell bank (MCB/WCB strategy; which is an off-the-shelf product approach).
- Step 6: Cell concentration.
- Step 7: Resuspension of the cells in formulation buffer.
- Step 8: Filling of the cell suspension in the primary container (vial or bag) and labelling of the primary container. This is considered the drug product (DP).
- Step 9: For some products, the cells are immediately shipped by a qualified courier to the side of administration after step 8. In such cases, the hospital should be at short distance, as the product cells are generally stable for hours to a couple of days at room temperature or at 2–8 °C (short term storage; non-off-the-shelf product). To allow for time between product manufacture plus quality control (QC) testing plus release of the DP and administration, and to allow for easy shipment to distant hospitals, the product is stored and shipped frozen, often in the vapor phase of liquid nitrogen at <–120 °C (long term storage).
- Step 10: Shipment of the DP to the clinical site.
- Step 11: Administration to the patient systemically (IV infusion) or locally with/without the use of a surgical procedure.

Figure 17.15 ■ Miltenyi's CliniMACs Prodigy closed processing system for cells grown in suspension (DCs, T cells)

Manufacturing of CAR-T Product

The manufacturing of genetically modified T cells is a multi-step process with slight modifications for each specific product (Fig. 17.18):

Figure 17.16 ■ Octane Technology, a fully closed production system for scale-out of autologous or allogeneic tissue- and cell-based products

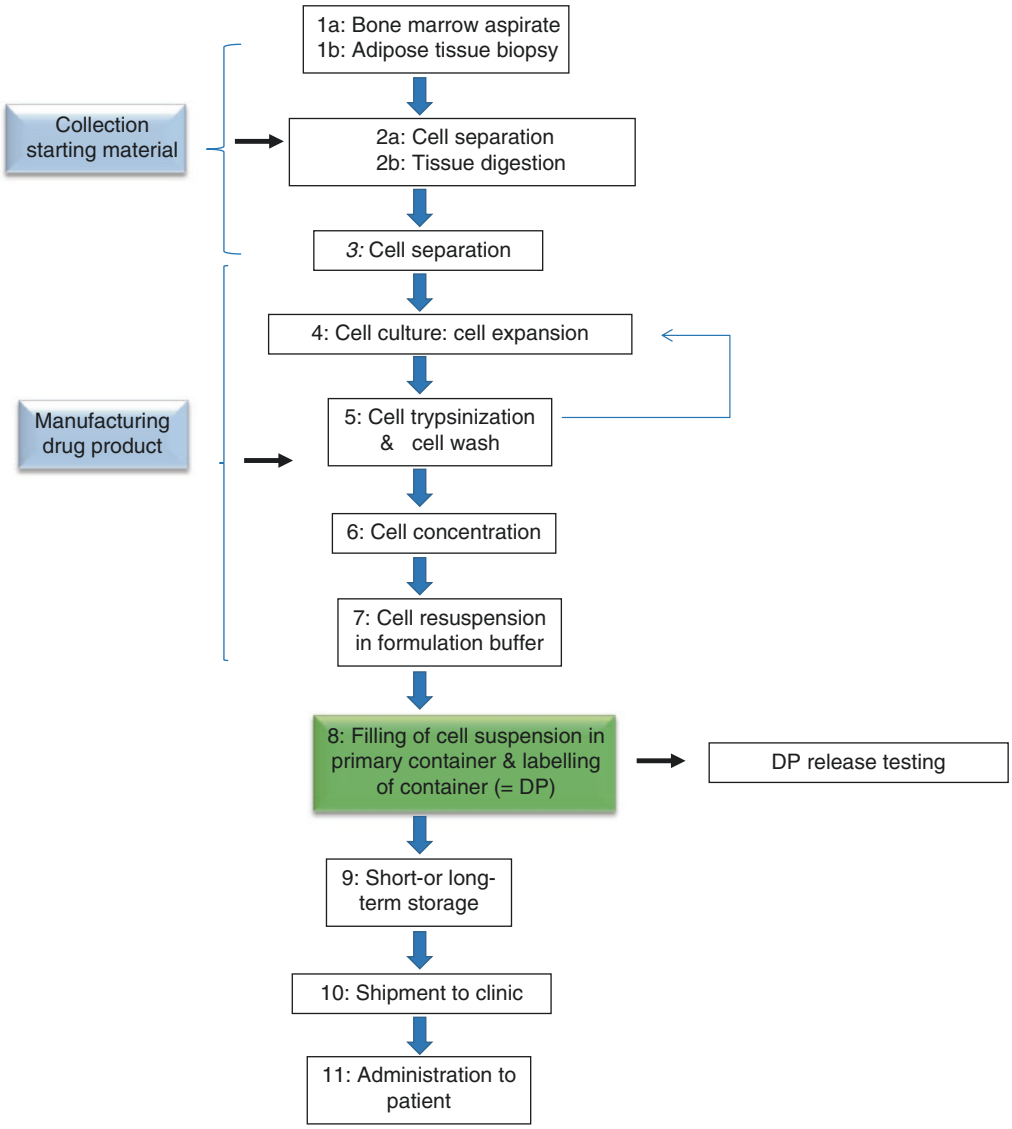

Figure 17.17 ■ Flow diagram of a manufacturing process for an off-the-shelf (allogeneic) or non-off-the-shelf (autologous or allogeneic) cell-based product based on adherent cells which do expand *ex-vivo*, such as MSCs

- Step 1: Harvest of blood cells by apheresis (whole blood collection) or leukapheresis (collection of leukocytes) from the patient (autologous cell source). The so called "starting material" is shipped either "fresh" (i.e., at room temperature or at 2–8 °C) or "frozen" (≤−80 °C) to the GMP manufacturing site. The patient is tested for specific human viruses prior to donation of the starting material.
- Step 2: From this starting material lymphocytes can be enriched either by counter-flow centrifugal elutriation or by subset selection according to the cellular phenotype.
- Step 3: The enriched lymphocyte population is placed in culture and stimulated with bead-based artificial antigen presenting cells (e.g., magnetic beads, coupled with mAbs).
- Step 4: The viral vector is added to transduce the genetic insert (CAR) into the T cells.
- Step 5: The cell culture is expanded in a bioreactor for several days until sufficient numbers of CAR-T cells are obtained for dosing and QC testing. The beads from step 3 are removed by a magnet as they are considered a process impurity.
- Step 6: The T cells are harvested, washed, and concentrated.
- Step 7: The cells are resuspended in the final product formulation buffer (7a) and filled in the primary container (infusion bag or vial). This is the so called "DP" (7b). Samples are taken for quality control testing.
- Step 8: For some products, the cells are immediately shipped by a qualified courier to the side of administration after step 7. In such cases, the hospital should be at short distance, as the product cells are

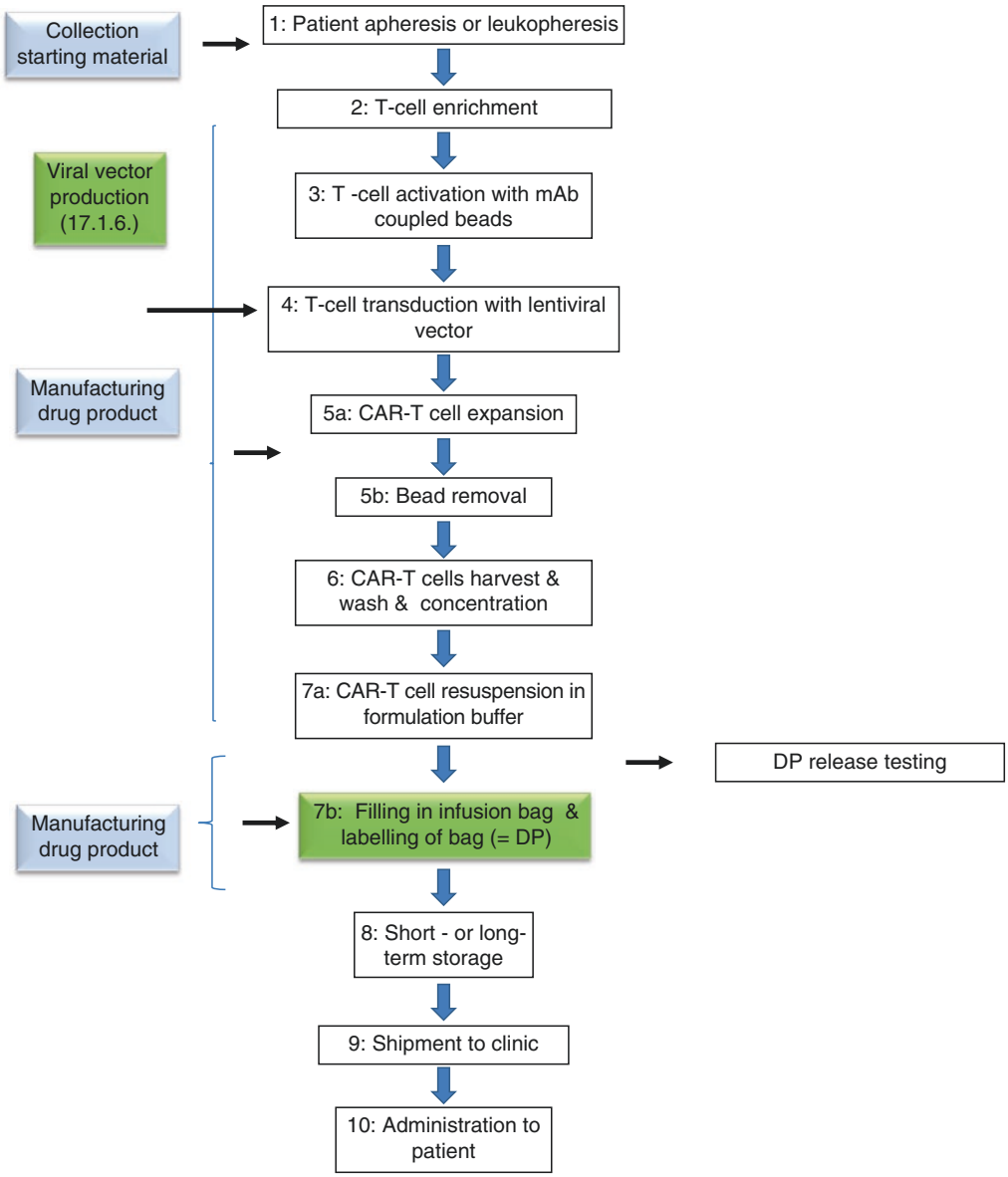

Figure 17.18 ■ Flow diagram of a CAR-T cell product manufacturing process. At the hospital white blood cells are harvested by leukapheresis (1). The starting material is shipped to the manufacturing facility for enrichment of the wanted T-cell populations (2); T-cell activation (3); transduction (genetic modification) of the T-cells with the lentiviral vector encoding the CAR genetic information (4). Thereafter, transduced cells (CAR-T cells) are *ex-vivo* expanded (5a) and purified via bead removal (5b). Cells are harvested, washed, and concentrated (6); cells are resuspended in formulation buffer (7a) and filled in the primary container (7b), which is labelled. This is considered the drug product. The product is stored (8) and thereafter shipped to the clinic (9). Prior to administration via IV infusion of the CAR-T cells at the hospital (10), the patient is pre-conditioned with chemotherapeutic medicines. Except steps 1 and 10, which take place at the hospital, all other steps take place at a manufacturing facility under GMP conditions. QC testing occurs between steps 1–2 (control of the starting material), in-process (steps 2–7a), and on the final drug product (step 7b)

generally stable for hours to a couple of days at room temperature or at 2–8 °C (short term storage). To allow for time between product manufacture plus QC testing plus release of the DP and administration, and to allow for easy shipment to distant hospitals, the product is stored and shipped frozen, often in the vapor phase of liquid nitrogen at < −120 °C (long term storage).

- Step 9: See step 10 manufacturing of MSC product.
- Step 10: At the site of administration the product is either administered directly to the patient or first thawed and sometimes washed to remove certain excipients such as dimethyl sulfoxide (DMSO) and then administered, often via IV infusion.

The chain-of identity of the entire process from leukapheresis to infusion and throughout all manufacturing steps vice versa (i.e., from donor to recipient and from recipient to donor) is controlled by a computer

based system to ensure the product's identity and product traceability.

Manufacturing of hESC Product

The manufacturing of a hESC derived combination product (cells in device) to treat DM type I is a multi-step process with expansion and complex differentiation steps, with slight modifications for each specific product (Fig. 17.19):

- Step 1: Isolation of the starting material (hESCs) via extraction of the inner cell mass from a 5-days old embryo (= blastocyte). This procedure can only take place after informed consent from the parent(s) and testing of the mother's blood for specific human viruses. This step does not take place at a manufacturing facility under GMP, but at an accredited tissue establishment, which is often a hospital.
- Step 2: Production of the pre-MCB by hESC culture initiation, cell expansion, cell wash, cell harvest, formulation of the cells in cryogenic medium, fill in a vial, and storage under cryogenic conditions in the vapor phase of liquid nitrogen.
- Step 3: Production of the MCB from a pre-MCB. A pre-MCB vial is thawed and cells are cultured expanded as described under "step 2" and followed by release testing of the MCB.
- Step 4: Production of a WCB from the MCB (see step 3) and release testing of the WCB.
- Step 5: A WCB vial is thawed and cells are expanded to obtain the required cell number for cell differentiation. Steps 2 through 5 take a couple of weeks.
- Step 6: Differentiation of undifferentiated hESCs into anterior definitive endoderm cells by adding specific growth factors and other factors to the culture medium. This step takes about 2 days.
- Step 7: Differentiation of anterior definitive endoderm cells into foregut endoderm cells by adding specific growth factors and other factors to the culture medium. This step takes about 3 days.
- Step 8: Differentiation of foregut endoderm cells into posterior foregut cells by adding specific growth factors and other factors to the culture medium. This step takes about 3 days.
- Step 9: Differentiation of posterior foregut cells into pancreatic endoderm cells by adding specific growth factors and other factors to the culture medium. This step takes about 4 days.
- Step 10: Pancreatic endoderm cells are harvested, washed, resuspended in cryo-preservation medium, and filled in cryovials. The cryovials are labelled. This is considered the "intermediate DP".
- Step 11: The intermediate DP is cryopreserved in the vapor phase of liquid nitrogen at < −120 °C (long term storage) and extensively QC tested prior to release of the intermediate DP.
- Step 12: Intermediate DP cryovials are thawed. In case steps 2 through 11 take place at a GMP facility on long distance from the clinical site where the drug product will be administered to the patient, the cryopreserved intermediate DP is shipped frozen to a GMP facility, often the hospital pharmacy, for preparation of the final drug product.
- Step 13: Intermediate DP cells are recovered from the freezing and thawing steps by placing them in culture for another 3–4 days.
- Step 14: The recovered cells are harvested and washed to remove dead cells and culture medium.
- Step 15: Cells are concentrated and formulated in a buffer.
- Step 16: Cells are uploaded into the immune-protective device using a loading device. The pancreatic pre-beta cells in the device is considered the DP. Limited QC release testing is performed on the DP.
- Step 17: The device is administered to the patient via a surgical procedure.

Key Factors for a Successful Manufacturing Process

To consistently manufacture advanced therapies at a large scale, automated manufacturing processes as well as the implementation of functionally closed systems are key success factors for the following reasons: (1) lower the risk of viral and bacterial contamination during manual and open process steps; (2) decrease costs associated with manual handlings; (3) improve product consistency; (4) shorten production times. Other key factors for success are: logistics around the manufacturing, supply chain of the product, and cost of goods. Particularly animal and human derived raw materials (for example growth factors, FBS), antibody coupled beads, and viral vectors are very expensive. Considering the high cost and increased risk of validating sterilization cycles of multiple-use bioreactors, these closed-processes for advanced therapies utilize single use, disposable bioreactors, mimicking current recombinant protein platform approaches (see Chap. 4). Despite some progress made in this field, there remains a requirement for better understanding of potential manufacturing platforms and how they can be best utilized for advanced therapies, taking the variety of cell and tissue types and clinical application into account.

Viral Vector Production for Ex-vivo Gene Modification of Cells

Recombinant viral vectors, e.g., retroviruses like lentiviruses, are produced by transfecting packaging cells, cultured with 3–4 plasmids that encode viral structural proteins (e.g., GAG, POL, Vesicular stomatitis virus (VSV)-G, and REV; the so called packaging plasmids) and the plasmid encoding the therapeutic gene of interest (e.g., CAR, ADA-SCID; the so called transfer plasmid). The transfer plasmid encoding the

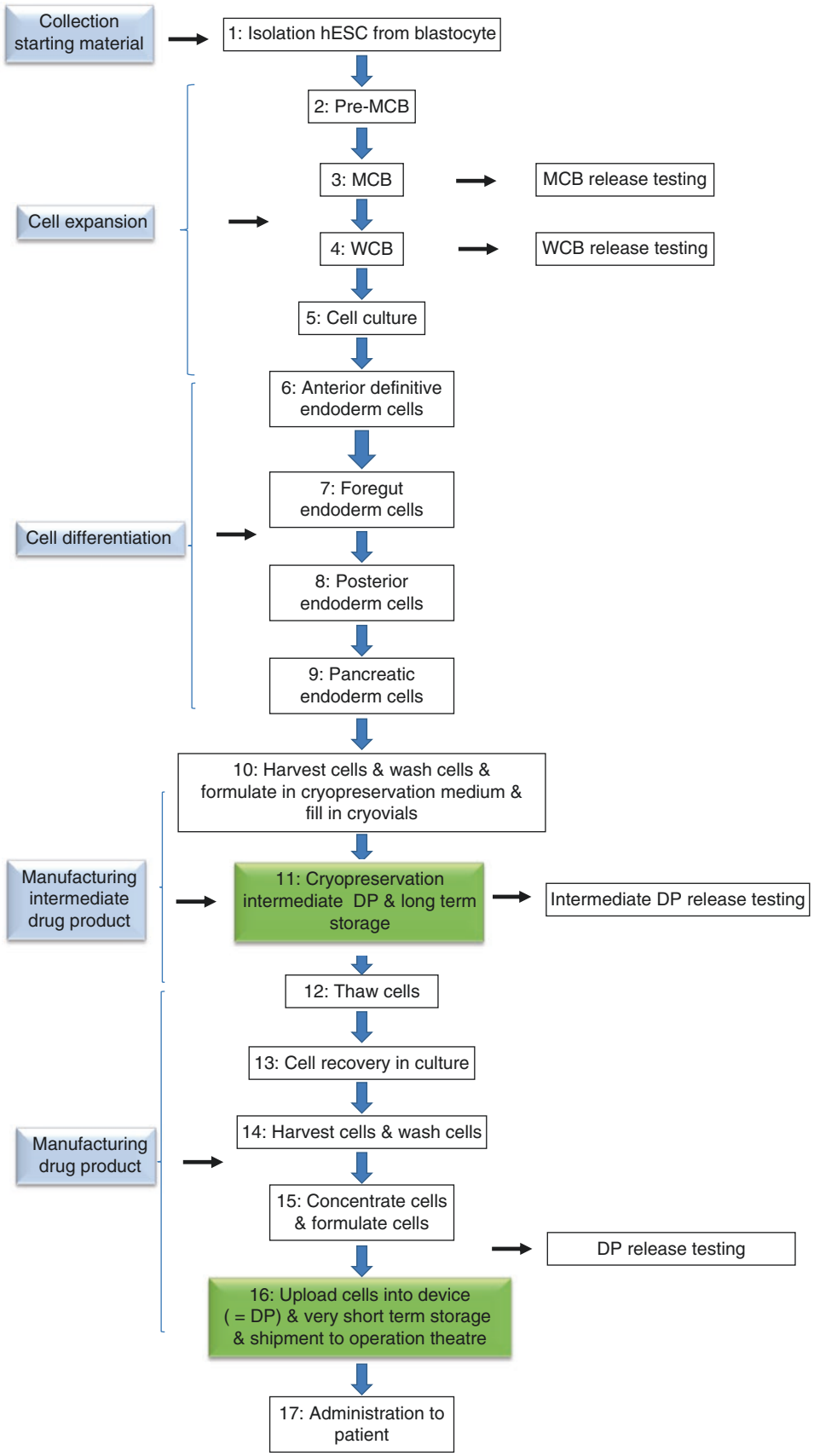

Figure 17.19 ■ Flow diagram of a hESC-derived combination product manufacturing process to treat DM type I

therapeutic gene contains the regulatory sequences that control its expression and a packaging sequence that enables its recognition. Within the packaging cell (e.g., the human embryonic kidney (HEK) 293 cell line), the RNA transcribed from the plasmid encoding the therapeutic gene is recognized by the viral proteins that assemble around it. The recombinant virus is then transported to the plasma membrane of the packaging cell that expresses viral envelope proteins (VSV-G). During budding the virus acquires the lipid bilayer from the packaging cell surface and incorporates the envelope proteins. The viral vector particles are released from the cells, which are cultured as adherent cells in culture flasks, into the cell culture medium. The above described steps are considered the upstream processing (USP) steps. From the medium the virus particles are subsequently harvested, formulated in a buffer and filled in the primary container. These production steps are considered the downstream processing (DSP) steps (Morenweiser 2005). DSP steps applied for viral vector production are steps traditionally used in the biotechnology industry for the manufacture of recombinant proteins. These are membrane-based (filt ration/clarification, concentration/diafiltration using tangential flow filtration, membrane-based chromatography) and chromatography based (ion-exchange chromatography, affinity chromatography, and size exclusion chromatography) process steps. The combination of these different process steps is variable and in some cases, different purification principles are used for the same purpose. Furthermore, a benzonase/DNase treatment for the degradation of contaminating DNA from the packaging cells is either part of the USP or DSP part of the manufacturing process. Subsequently, the purified virus particles are formulated in a buffer, filled in the primary container, stored frozen, and tested until further use for transduction of the cells to make a genetically modified cell therapy product (see Wright 2018). Figure 17.20 provides a schematic overview of the entire viral vector material manufacturing process used in the production of a genetically modified cell therapy product. For production of a viral vector product for *in-vivo* gene therapy (see Chap. 16), the production process is identical.

■ Excipients

Common excipients used in the formulation of advanced therapies are presented in Table 17.13. Most of these excipients overlap with those used in therapeutic protein products. However, KCl, $MgCl_2$, nucleosides, FBS, and DMSO are not found in therapeutic protein drug products.

Table 17.14 provides an overview of a few commercially available advanced therapies with their formulation and shelf-life.

■ Primary Container

Generally, two types of containers are used for cell-based products: vials (small volume, low dose), and infusion bags (higher volume and dose), as shown in Fig. 17.21. Tissue-based products often have a non-standard container for storage and shipment.

■ Storage and Shipment

Stability of the starting material (cells or tissue and viral vector) and DP are an important element for successful production, storage, shipment, and administration of advanced therapies. Starting materials and DPs either have a very short shelf-life of hours–days and are stored and transported at 2–8 °C or at room temperature or have a longer shelf-life (months–years) and are stored and shipped frozen (cryopreserved in the vapor phase of liquid nitrogen at <−120 °C or in a −80 °C or −150 °C freezer).

■ Manufacturing Model: Scale–Up Versus Scale–Out

Broadly speaking, there are two paradigms in advanced therapy manufacture: off-the-shelf (always allogeneic source of cells/tissue) and patient specific (commonly autologous source of starting materials, but sometimes allogeneic) DPs. Off-the-shelf products represent a business model akin to current biopharmaceuticals, where one batch can be manufactured to treat multiple patients. This allows for increasing economies of scale, which drives down the per-dose cost of the final product. This means that there is a wealth of engineering and process knowledge and technologies that can be leveraged to support the manufacture of off-the-shelf advanced therapies at increasing scale.

However, scale-up is not just about making the reactor growing the cells bigger. Conventional scale-up bioprocesses typically use cells to produce therapeutic agents (e.g., mAbs), which can then be isolated and purified without the need to recover the cell. For the manufacture of advanced therapies, where the cells/tissue culture is the product of interest, retention of cell viability, phenotype, and function to assure quality, is of primary importance in order to preserve product safety and efficacy. As the number of cells increase during expansion this can become increasingly challenging, as the greater cell numbers lead to an increased chance of inhomogeneity of culture and hence of cellular performance being altered. This means that the desired quality of the cells/tissue must be maintained through the entire manufacturing process, including the harvest and DSP, storage, shipment, and delivery to the patient. This will require the development of scalable harvesting, DSP, and formulation technologies to cope with the large batch size produced.

Patient-specific advanced therapies offer a new challenge for process scalability, where the manufacturing process must be scaled-out, in order to pro-

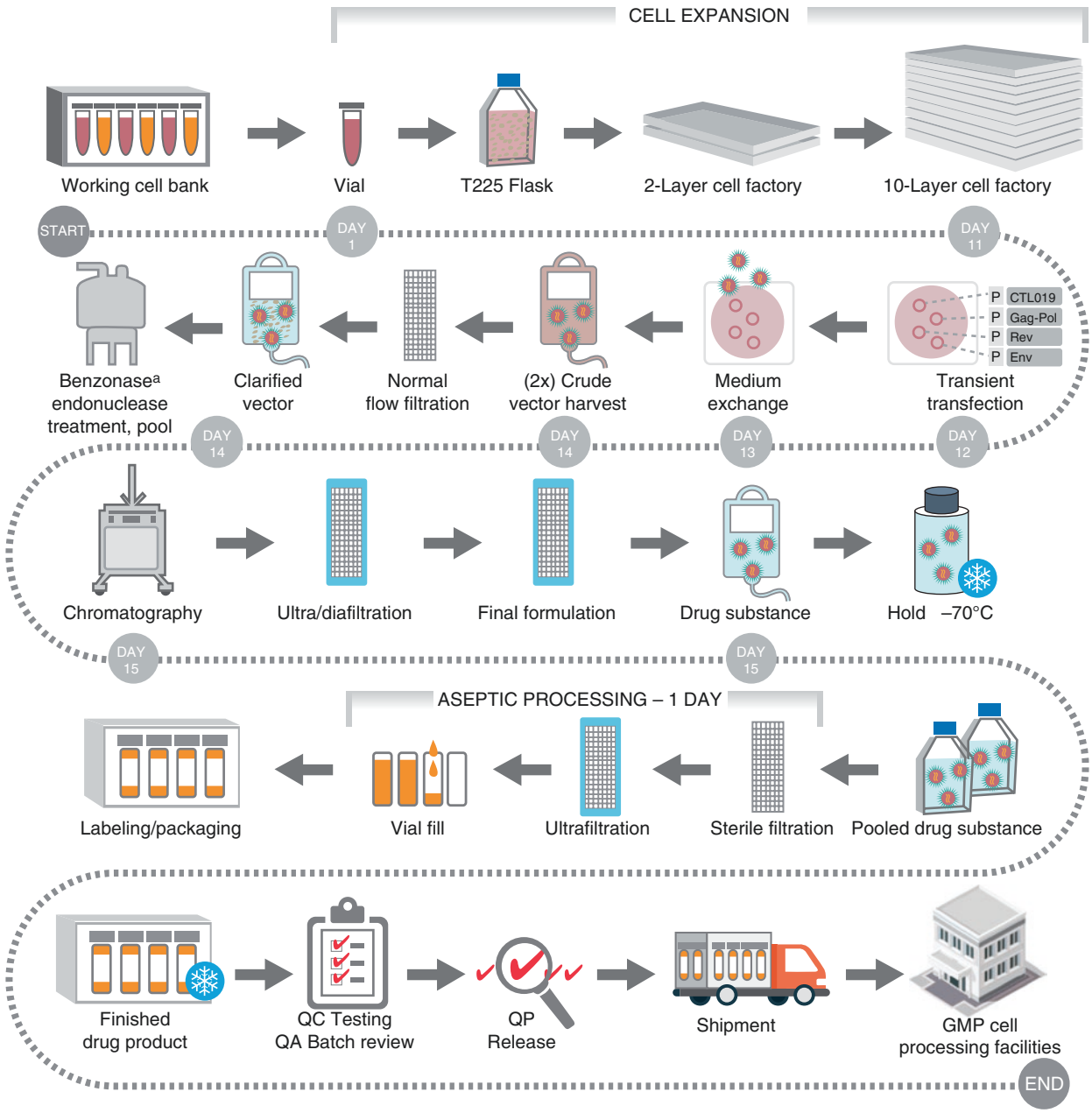

Figure 17.20 ■ Schematic overview of a lentiviral vector manufacturing process. The produced viral vector is used as starting material for the genetic modification of T-cells in the manufacture of a CAR-T product, reprinted with permission (Levine et al. 2017). A similar production approach is taken for other *ex-vivo* gene therapy as well as *in-vivo* gene therapy products (cf. Chap. 16). *QC* = quality control, *QP* = qualified person, *QA* = quality assurance

duce one batch for each patient (Fig. 17.22). This introduces the concept of "personalized medicine" (see Chap. 9), where the cost of production per batch cannot be reduced by exploiting an increasing economy of scale by simply producing a larger batch. Reducing the cost of these patient-specific cell- and tissue-based products must therefore be achieved by advances in engineering and manufacturing technology, reducing the number of complex, labor-intensive, and open-process steps that are commonplace in manufacture of these products at research labs. The developments of closed and automated processes as well as process simplification are key factors for commercial success as this will allow multiple batches to be produced in parallel (scale-out), with reduced burden of oversight by highly-trained scientists. These new processes must be GMP-compliant and closed for sterility.

■ Testing

As for any DP, cell- and tissue-based therapies are subject to detailed characterization. This involves assessment of quality attributes (i.e., identity, purity and impurities, viability (Cadena-Herrera et al. 2015), bio-

activity (potency; Bravery et al. 2013), safety, quantity, and general attributes, such as appearance, pH, morphology) both of the cellular/tissue/vector starting material and the final DP, see Table 17.15. The latter includes QC testing to allow release of the DP for administration. In addition, at different stages of the production, in-process controls are performed to assess the quality and stability of the cells/tissue during manufacture. A sub-set of characterization tools is used for assessment of stability of the starting material(s) and DP.

Excipients class	Function	Example
Buffer	pH stabilizer	TRIS, histidine, Na-acetate
Salt	Stabilizer	NaCl, KCl, $MgCl_2$
Antioxidant	Prevent oxidation	Methionine
Sugar	Stabilizer, cryoprotectant, tonicity modifier	Mannitol, trehalose, sucrose, glucose
Polyol	Collapse temperature modifier	Dextran (low and high molecular weight)
Nucleoside	Stabilizer	Adenosine, guanosine
Protein	Stabilizer, preservative	Fetal bovine serum, human serum albumin
Organic solvent	Stabilizer, cryoprotectant, solvent	Glycerol, ethylene glycol, DMSO

Table 17.13 ■ Examples of excipients used in the formulation of advanced therapy products

However, for a lot of autologous and some allogeneic DPs that are not "off-the-shelf", performing QC tests may be challenging due to the time constraints between manufacture and administration, i.e., the shelf-life of the drug product is hours–days. Moreover, for some autologous products all the available cell/tissue material is needed for the dose. In such cases, product release may be justified by extensive process validation; in-process control testing and/or QC testing data becoming available after product administration. These approaches require a paradigm shift in the pharma world, where traditionally products are only administered after extensive testing and batch release.

Adequate QC of starting materials such as cells/tissue biopsy and viral vectors is crucial as poor quality starting material will affect the quality of the final product. Autologous or allogeneic cells/tissue can be very heterogeneous due to the inherent donor variability (age, sex, health status, medication), the variable amount of cells other than the intended cells, and because the collected cells are not in a synchronized cell cycle. In addition, the origin of the cells (e.g., MSCs of bone marrow, adipose, and cord blood origin) may have significant impact on the activity and phenotype of the cells after manufacture.

The challenge is that a lot of the techniques used for the characterization of this heterogeneous group of products are not sensitive methods, hence they are not able to pick-up subtle changes to the process and/or to the product.

For further reading on characterization of cell- and tissue-based products, see BSI PAS 93:2011.

Product	Shelf-life and storage condition	Composition (active substance)	Excipients/mixtures
Provenge® Suspension of cells for IV infusion	18 h at 2–8 °C	≥50×10^6 autologous $CD54^+$ cells/250 mL activated with PAP-GM-CSF[a]	Lactated Ringer's solution (NaCl, $NaC_3H_5O_3$, KCl, $CaCl_2$)
ChondroCelect® Suspension of cells for implantation	48 h at 15–25 °C	4×10^6 autologous human cartilage cells/ 0.4 ml	DMEM[b]
MACI® Implantation matrix plus cells in solution for implantation	6 days at ≤37 °C and keep out of fridge	0.5×10^6 to 1×10^6 autologous cultured chondrocytes/cm^2 porcine derived type I/III collagen membrane	DMEM, HEPES[c] adjusted for pH with HCl or NaOH and osmality with NaCl
Kymriah® Suspension of cells for IV infusion	9 months at ≤−120 °C in the vapor phase of liquid nitrogen	2×10^6–2.5×10^8 autologous CAR-positive viable T cells	Plasmalyte-A[d], glucose/NaCl, human serum albumin, dextran 40-low molecular weight/glucose, DMSO

[a]Prostatic acid phosphatase granulocyte-macrophage colony-stimulating factor
[b]Calcium Chloride anhydrous, Ferric Nitrate.$9H_2O$, Potassium Chloride, Magnesium Sulphate anhydrous, Sodium Chloride, Sodium Bicarbonate, Potassium Phosphate Monobasic.H_2O, D-Glucose, L-Arginine.HCl, L-Cystine.2HCl, L-Glutamine, Glycine, L-Histidine.HCl.H_2O, L-Isoleucine, L-Leucine, L-Lysine.HCl, L-Methionine, L-Phenylalanine, L-Serine, L-Threonine, L-Tryptophan, L-Tyrosine.$2Na.2H_2O$, L-Valine, D-Calcium Pantothenate, Choline Chloride, Folic Acid, i-Inositol, Niacinamide, Riboflavin, Thiamine.HCl, Pyridoxine.HCl
[c]4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid sodium
[d]Plasmalyte-A sodium chloride: 5.26 g/l potassium chloride: 0.37 g/l magnesium chloride hexahydrate: 0.30 g/l sodium acetate trihydrate

Table 17.14 ■ Examples of approved advanced therapies, their formulation, and shelf-lives

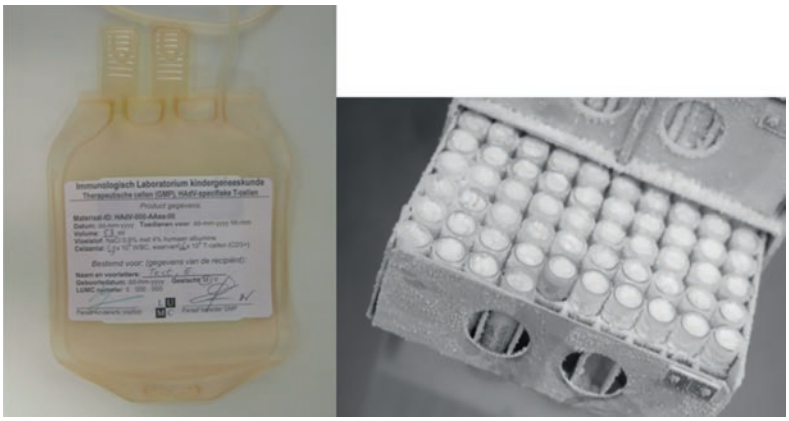

Figure 17.21 ■ Examples of primary containers for the storage and transport of advanced therapies. Left photo: infusion bag; right photo: cryovials in box to allow for storage in the vapor phase of liquid nitrogen (courtesy of M. de Haan)

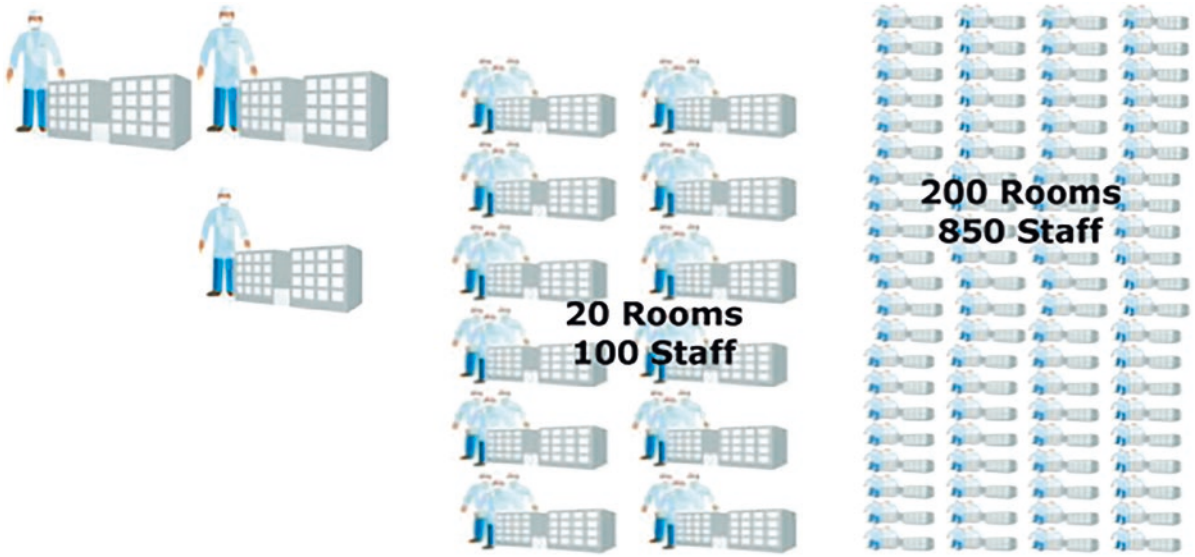

Figure 17.22 ■ Scale-out of a labor intensive manual process

For details on testing (lot release and additional characterization) of viral vectors for *ex-vivo* and *in-vivo* gene therapy products, see Gombold (Gombold et al. 2006a, b); Wright (2018).

Table 17.16 provides an overview of the QC testing panel for an MSC–derived and a CAR-T product.

Flow Cytometry

One of the key technologies in advanced therapy manufacturing is flow cytometry. It can be operated in a QC test environment and in production of advanced therapies products (see next section). As this technique is not used regularly to characterize therapeutic proteins, it is not discussed in Chap. 3. We pay attention to it in this chapter.

Flow cytometry assays may be used to assess cell- and tissue-based product identity, active substance purity, cellular impurity, viability, and potency testing. It is a powerful technique, which allows for specific measurement of cellular components on the cell surface (e.g., CD73, CD90, and CD105 to characterize MSCs) and intracellular components. It is also amenable to the measurement of soluble analyte(s) such as cytokines, released by the cells in the extracellular environment, e.g., upon cell activation.

Flow cytometry is a technology that simultaneously measures and then analyzes multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence. A flow cytometer is made up of three main systems: fluidics, optics, and electronics.

- The fluidics system transports single particles (cells) in a stream to the laser beam for interrogation.
- The optics system consists of a light source, mostly lasers, to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors. Light scattering or fluorescence emission from auto-fluorescence of the particle or from fluorophores, which are fluorescence labels (e.g., bound to specific anti-

Quality attribute	Explanation	Possible techniques applied
Identity	Distinguish the cellular active substance (s)/tissue from unwanted cell population(s); donor specific test; sometimes a combination of tests	Flow cytometry[g]; karyology, STR[a], FISH[b], CGH[c], microscopy, immunocytochemistry, electrochemiluminescence, protein array, microarray
Active substance purity	Number of viable cells with specific cell surface markers present/absent, unique for the active substance. Closely related to identity	Flow cytometry; ELISA[d]; immunocytochemistry; electrochemiluminescence; protein ligation assay
Cellular (product) impurities	Dead cells (based on total and viable cell numbers); unwanted cell populations. Closely related to identity and purity	Flow cytometry; ELISA; electrochemiluminescence; MS[e]
Process impurities	Depends on process and raw materials used, e.g. antibiotics, cytokines, growth factors, FBS, beads, viral vector starting material	– Cytokines, growth factors, FBS, TryPLESelect: ELISA – Beads: microscopic evaluation; – Antibiotics: LC-MS[f]; – Viral vector: qPCR[h]
Potency/ bioactivity	Quantitative measure of relevant biologic function(s) based on the attributes that are linked to relevant *in-vivo* biologic properties; often a combination of assays. Receptors, cellular metabolism, secreted proteins, migration of cells, (lack of) proliferation, differentiation potential, mRNA expression	ELISA; qPCR; flow cytometry; cell migration in Dunn or Boyden chamber; protein array; LC; MS; animal model (not quantitative), microarray
Viability and total cell count	Viability is a critical parameter and related to dose, purity and cellular impurities	Colorimetric assay (spectrophotometer), fluorescent assay (including flow cytometry), membrane integrity assay (e.g., trypan blue), microscope. Manual, semi-automated or automated equipment
Dose	Often number of total or viable cells per unit (mL, kg body weight); cm^2 of tissue	Total cell count and viability techniques
Safety	Sterility, endotoxin, mycoplasma, human and animal viruses derived from starting material or raw materials, replication competent viral vector, chromosomal aberrations	Pharmacopoeial tests for sterility, mycoplasma, endotoxin-standard or rapid tests; chromosomal aberrations by karyology, FISH, CGH
General attribute	Appearance, pH, osmolality, particles, cell/tissue morphology	Pharmacopoeial tests, microscope for morphology assessment

[a]*STR* = short tandem repeat
[b]*FISH* = Fluorescence in situ hybridization
[c]*CGH* = comparative genomic hybridization
[d]*ELISA* = Enzyme-Linked Immuno Sorbent Assay; see Chap. 3 for details on this technique
[e]*MS* = mass spectrometry
[f]*LC-MS* = liquid chromatography-mass spectrometry; see Chap. 3 for details on this technique
[g]Flow cytometry technique is explained below; it can be used for intracellular and cell surface markers
[h]*qPCR* = quantitative polymerase chain reaction; see Chap. 1 for details on PCR

Table 17.15 ■ Examples of techniques applied for the analysis of different quality attributes of cell- and tissue based therapies. Some techniques are also used for starting material characterization

bodies) used to detect the expression of cellular molecules such as specific proteins or nucleic acids) provides information about the particle's properties. (1) Light that is scattered in the forward direction after interacting with a particle, typically up to 20° offset from the laser beam's axis, is collected by a photomultiplier tube or photodiode and is known as the forward scatter (FSC) channel. This FSC measurement can give an estimation of a particle's size with larger particles refracting more light than smaller particles. (2) Light measured at a 90° angle to the excitation line is called side scatter (SSC). The SSC can provide information about the relative complexity (e.g., granularity and internal structures) of a cell or particle; however, as with forward scatter this can depend on various factors. Both FSC and SSC are unique for every particle and a combination of the two can be used to roughly differentiate cell types in a heterogeneous population such as blood or bone marrow aspirate. However, this scatter information and cell typing depends on the sample type and the quality of sample preparation, so fluorescent labelling is generally required to obtain more detailed information.

– The electronics system converts the detected light signals into electronic signals that can be processed by the computer.
– In the flow cytometer, particles are carried to the laser intercept in a fluid stream. Any suspended particle or cell from 0.2–150 µm in size is suitable for

Quality attribute	MSC derived cell based product; allogeneic off-the-shelf (1 batch of multiple vials/bags for multiple patients)	CAR-T *ex-vivo* gene therapy product; autologous (1 batch of 1 infusion bag for 1 patient)
Identity	$CD73^+$, $CD90^+$, $CD105^+$, HLA-DR^-, $CD3^-$, $CD45^-$ cells by flow cytometry	CAR expression by qPCR
Viability by manual or automated cell count	Number of total cells	Number of total cells
	Number of viable cells	Number of viable cells
	Percentage of viable cells	Percentage of viable cells
Purity by flow cytometry (% of viable cells with a certain CD-marker profile)	Percentage of $CD73^+$, $CD90^+$, $CD105^+$, 7-AAD^- cells by flow cytometry	Percentage of viable T cells
		Transduction efficiency by CAR q-PCR
Product = cellular impurities (dead cells and unwanted cell populations) by flow cytometry	Percentages of 7-AAD^+ (dead cells), $CD3^+$ (T cells), $CD45^+$ (lymphocytes), $CD34^+$ (HSCs and endothelial cells), $CD14^+$ (monocytes), $CD19^+$ (B cells)	Percentages of red blood cells, granulocytes, dead cells, $CD19^+$ B cells
Process impurities	Residual bovine serum albumin (BSA) by ELISA	Residual antibody conjugated beads (CD3/CD28)
	Residual TryPLESelect by ELISA	BSA by ELISA
	Residual antibiotic by liquid chromatography-mass spectrometry	Residual VSV-G DNA by qPCR-derived from viral vector
Potency	CD marker expression (e.g., adhesion molecules) upon immune activation by flow cytometry	Determination of CAR expression by flow cytometry
		Release of interferon-gamma in response to CD19-expressing target cells
Safety	Sterility	Sterility
	Bacterial endotoxins	Endotoxin
	Mycoplasma	Mycoplasma
	Karyology	PCR-based replication competent lentivirus assay
	Human viral testing; test for the presence of inapparent virus; *in-vitro* assay for the presence of viral contaminants	N.A.
Dose (calculated)	a–b × 10^6 viable $CD73^+$, $CD90^+$, $CD105^+$, 7-AAD^- cells/ml	a–b × 10^6 $CD19^+$ T cells/kg body weight
General attribute	pH	pH
	Osmolality	Osmolality
	Appearance of primary container and content	Appearance of primary container and content
	Content uniformity	N.A.
	Extractable volume from the vial	N.A.

Table 17.16 ■ Example of QC testing panel for an MSC-derived cell based product and a CAR-T *ex-vivo* gene therapy product

analysis. Cells from solid tissue must be desegregated into single cells before analysis. The portion of the fluid stream where particles are located is called the sample core. When particles pass through the laser intercept, they scatter laser light. Any fluorescent molecules present on the particle fluoresce. The scattered and fluorescent light is collected by appropriately positioned lenses. A combination of beam splitters and filters steers the scattered and fluorescent light to the appropriate detectors. The detectors produce electronic signals proportional to the optical signals striking them. Read outs are collected on each particle or single event. The characteristics or parameters of each event are based on its light scattering and fluorescent properties. The data are collected and stored in the computer. This data can be analyzed to provide information about sub-populations of cells within the sample (see Fig. 17.23).

Figure 17.24 provides representative flow cytometry histograms for an MSC product.

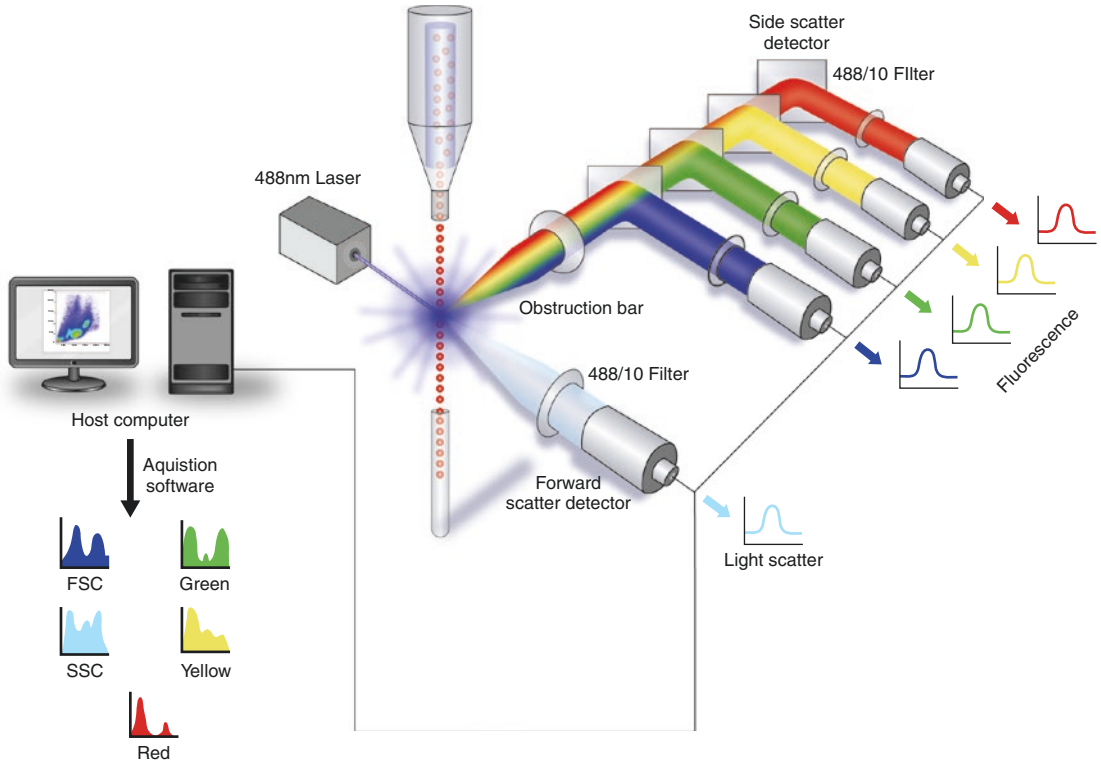

Figure 17.23 Schematic view of a flow cytometer. Scattered and emitted light signals are converted to electronic pulses, adapted from ThermoFisher Scientific. http://www.thermofisher.com/nl/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/flow-cytometry-basics/flow-cytometry-fundamentals/how-flow-cytometer-works.html#overview

Fluorescence-Activated Cell Sorting (FACS)

Flow cytometry techniques can also be used for sorting of specific cell (sub) populations, e.g., to increase product yield and/or to reduce the amount of unwanted cell populations, which are considered impurities. A FACS machine provides the ability to separate cells identified by flow cytometry. Droplet based cell sorters first analyze the particles but also have hardware that can generate droplets and a means of deflecting or directing wanted particles into a collection tube. Cell dispersions are often purified based on surface markers such as CD34+ in HSCs or on their viability. Common uses of cell sorting include identifying and isolating cell populations or single cells followed by subsequent downstream applications where DNA, protein or cellular function is investigated.

Improvements of Testing Strategies Needed

Developing robust, sensitive, rapid, and in-line analytical testing and characterization tools will be required as cell/tissue and viral vector processing platvforms continue to evolve. Significant improvements are needed to establish next-generation analytics for (in-process) QC, stability, and additional characterization testing to assess the quality attributes of starting materials, intermediates, and advanced therapy products. Improvements are also to be made in the field of in-line and on-line testing of cell culture conditions (e.g., pH, morphology and viability). Reducing the sampling frequency, technical complexity, amount of sample needed, and labor intensiveness of testing is especially critical for a non-off-the-shelf autologous *ex-vivo* gene therapy product. This contrasts with traditional biopharmaceuticals where a single batch of QC tested product may treat hundreds or thousands of patients. Cell processing automation will also be enabled through the development of high throughput in-process and release assays providing results in a very short timeframe (minutes–hours). Advanced cell/tissue characterization techniques based on nanofluidics, transcriptomics, and proteomics, and next generation sequencing techniques may allow better understanding of what happens to desired cell population(s)/tissue once they are processed and before patient administration (see Chap. 9 for more details on "-omics"), both in the cytosol as well as in the extracellular environment. Examples are changes in intracellular genetic profiles and patterns within the micro RNA and exosome pools secreted into the culture medium by the cells.

Different advanced therapy technologies are currently at different stages in translation and do have their particular manufacturing and testing challenges, as summarized in Table 17.17.

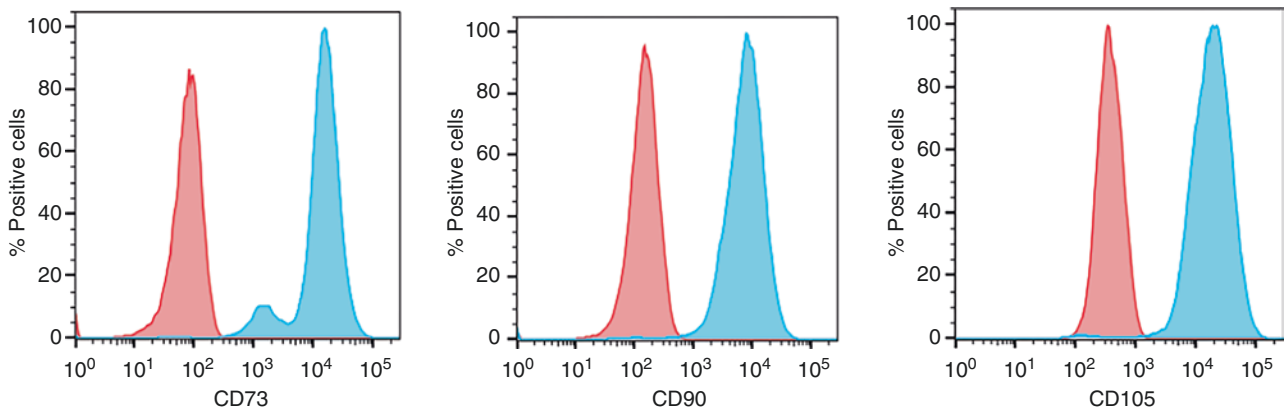

Figure 17.24 Flow cytometry histograms of MSC product cells. Flow cytometric analysis of MSC product cells against three defined MSC markers (CD73, CD90, and CD105) show that these cells are of mesenchymal cell phenotype. On the X-axis the density of the respective cell surface marker molecule is shown. A single peak is observed for each of the markers tested (blue peak at the right side of each histogram), indicating a single population of cells. The red peak at the left side of each histogram represents the isotype control staining. Courtesy of M. van Pel

OTHER ASPECTS OF ADVANCED THERAPIES

■ Regulatory Bodies Involved in Regulating Advanced Therapies in Europe

In Europe, the responsibility for regulating transplant products according to the public health legislation lies with the national Competent Authority for tissues and cells in each member state. ATMPs in contrast are regulated by the pharmaceutical legislation, hence a marketing approval must be obtained prior to the marketing of an ATMP through the centralized procedure, like for any other biological medicinal product. The scientific evaluation of these products is led by a specialized committee within the EMA (the Committee for Advanced Therapies—"CAT"). The CAT drafts an opinion for the Committee for Medicinal Products for Human Use (CHMP), which is responsible for providing a second scientific opinion. Based on a positive CHMP opinion, the approval of a marketing authorization application (MAA) is granted by the EC. Clinical trials involving ATMPs are regulated and authorized in the same manner as other medicinal products, i.e., on a national level by the appropriate national competent authority (NCA).

■ Regulatory Bodies Involved in Regulating Advanced Therapies in the USA

The situation in the US is simpler in that the FDA is responsible for both aspects of the legislation: the public health and the pharmaceutical legislation. Within the FDA, the responsibility for the regulation of HCT/Ps and human gene therapy products lies with the Center for Biologics Evaluation and Research (CBER), both for clinical trials and marketing authorization. As of 2016, the CBER structure includes the Office of Blood Research and Review (OBRR), the Office of Vaccines Research and Review (OVRR), and the Office of Tissues and Advanced Therapies (OTAT), which was formerly known as the Office of Cellular, Tissue and Gene Therapies (OCTGT). To monitor activity, review data, and anticipate future needs, the FDA operates the Cellular, Tissue and Gene Therapies Advisory Committee.

■ Regulatory Guidances

Links to the relevant regulatory bodies involved in advanced therapies in the EU and US as well as applicable guidances can be found in Table 17.18.

■ Stem Cell Tourism

The general interest in advanced therapies around the world has allowed unregulated practice particularly of cell-based products to develop in some countries, i.e., "stem cell tourism". This is a major concern for many stakeholders in the field of ATMPs, because treatments are being offered in the absence of a strong safety data package and any proven efficacy. In addition, there is suspicion that the products in use have been manufactured with insufficient attention to GMP including quality control. It is very important that patients are warned of the dangers of falling prey to unethical operations. An up-to-date source of information on private clinics and stem cell tourism is available at the website of the International Society for Stem Cell Research (www.isscr.org).

CONCLUDING REMARKS

Although progress has been made in the area of ATMPs, with about 30 products approved globally and 20 in the EU&US (see Table 17.1) for commercial use and many products in clinical development, this field is currently struggling with similar problems as the first recombinant proteins 20 years ago. Appropriate manufacturing platforms, supply chain models, healthcare systems, reimbursement models, and regulatory frameworks for these medicinal products need to be established by developers and other key stakeholders, while specific knowledge about quality (production and testing), safety, and efficacy of advanced therapies is steadily growing.

Technologies	Development stage of the field	Current manufacturing technologies	Manufacturing and testing challenges
(a) Somatic cell technologies	Many products in early clinical development phase; few products approved, e.g., Alofisel	Manual process with open handling steps; automated multi-planar flasks and stack systems; micro-carriers in disposable stirred tank systems; hollow fiber growth systems; membrane and contraflow centrifugation systems	Scale-up and control of large scale batches. Recovery of cells from micro-carriers. DSP: Large volume handling, primary container filling at scale using enclosed technologies. Relevant potency assays lacking
(b) Cell immortalization technologies	One product in early clinical development	CompacT SelecT[a] fully automated and programmable scalable cell culture platform consisting of a robot arm that can access T175 flask or multi-well plate incubator. Standard cell culture activities, such as passage or media change, are conducted and controlled with no manual intervention	Similar to protein manufacturing platform technologies
(c) *Ex-vivo* gene modification of cells using viral vector technologies	Mainly small trials in early and late clinical development phase (gene modified autologous T-cells and HSCs); few products approved, e.g., Strimvelis and Kymriah	Manual processes often not fully enclosed using static bags, gas-permeable pots + lateral movement bioreactors (wave bags) for higher cell yield. Positive or negative cell selection process steps often used. High cell purity becoming possible with sterile cell sorter	Adapting systems to deal with variation in quality and amount of incoming starting material. Lack of product stability pressuring manufacturing and distribution model. Lack of fast QC assays. Low transduction efficiency with non-replicating viral vectors. Enclosed and automated manufacturing systems are becoming available for the entire process (e.g., prodigy)
(d) Cell plasticity technologies	Mainly pre-clinical phase with few ESC and iPSC-derived FIH trials	Current processes are extremely 'manual' and rely on small scale cell culture and harvest technologies. High risk processes with extensive process and product characterization testing to assess product quality, safety, and efficacy	A two-tier banking strategy (MCB/WCB) scale-up process of pluripotent cells prior to differentiation steps needed. Dynamic cell culture systems to expand PSC numbers. Robotic scale-out of current plate-based iPSC technology is also being explored
(e) 3D-technologies	Mainly pre-clinical phase with few FIH trials	A complex manufacturing interplay between (bio)materials, scaffolds, cells, and biological coatings. Incorporates decellularization/recellularization tissue-based products such as trachea, esophagus, and veins	Enclosed bioreactors to control cell and material interface. Improved stability and delivery systems. Robust product quality to ensure large clinical application

[a]Thomas et al. (2009)

Table 17.17 ■ Development stage manufacturing and testing challenges for different advanced therapy technologies, adapted from Mount et al. (2015)

Regulatory agency/institute	Link
EMA	http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000294.jsp&mid=WC0b01ac05800241e0
FDA	https://www.fda.gov/BiologicsBloodVaccines/default.htm
International Conference on Harmonisation (ICH)	http://www.ich.org/home.html
British Standards Institution (BSI)	BSI PAS 83:2012, BSI PAS 84:2012, and BSI PAS 93:2011

Table 17.18 ■ Regulatory agencies and applicable guidances for advanced therapies in the US and EU

This rapidly evolving field does require highly trained practitioners, both at the technical level and with regard to advising and counseling patients. Pharmaceutical scientists and pharmacists are important members of the teams of professionals that deliver these changes in healthcare to seriously ill patients. Much can be learned from the research & development (R&D) processes used by traditional biotech (e.g., during development of recombinant proteins and vaccines). Pharmaceutical scientists and pharmacists can play a key role in development of advanced therapies, as many applications are conceived by academic groups and small spin-off companies, who do not necessarily know how to translate a research concept into a medicinal product for human use. Pharmacy professionals can provide valuable experience in relation to the application of the principles of GMP, GLP, GCP, GDP (good distribution practice), and other available guidances.

SELF-ASSESSMENT QUESTIONS

■ Questions

1. What is the difference between embryonic and adult stem cells?
2. How is somatic cell nuclear transfer carried out and what are the problems with this technique?
3. What are iPSCs and why are they important?
4. What is the difference between *in-vivo* gene therapy and *ex-vivo* gene therapy?
5. Which disease areas are predominantly investigated clinically with ATMPs?
6. What problems could arise in use of stem cell-derived products for clinical application?

■ Answers

1. Embryonic stem cells are grown *ex-vivo* after extraction of the inner cell mass from a blastocyst. Adult stem cells are found *in-vivo* in many tissues, usually in the specialized environment of a stem cell niche, that supports their asymmetric cell division.
2. Somatic cell nuclear transfer (SCNT) involves the injection of a donor genome into an enucleated egg, such that the embryo develops as a clone of the donor genome. This allows the generation of embryonic stem cells using the donor genome and, in principle, allows implantation into the uterus of a recipient female leading to pregnancy. There are ethical problems concerned with supply of fertilized human eggs and also technical problems caused by incomplete reprogramming of the donor nucleus.
3. iPSCs are produced by transient expression of pluripotency genes in somatic cells, leading to reprogramming to form pluripotent cells resembling embryonic stem cells. The production of iPSCs allows pluripotent cells to be obtained from a patient without the need for SCNT. iPSCs can be used to derive differentiated cells for the production of ATMPs for clinical application or for disease modeling purposes.
4. *In-vivo* gene therapy refers to the direct introduction of genetic material into the human body, whereas *ex-vivo* gene therapy refers to the use of cells, which are genetically modified outside the body (i.e., *ex-vivo*) prior to administration of these genetically modified cells into the human body. In the latter case, the genetic material is introduced into the human body using cells as "delivery system". See also Chap. 16 for details on gene therapy.
5. Various cancers, autoimmune disorders, such as DM type I and Crohn's disease, neurological disorders, such as Parkinson's disease and Alzheimer's disease, myocardial infarction, and macular degeneration.
6. One of the concerns with stem cell derived ATMPs is the possibility that rare pluripotent or multipotent cells in the product could give rise to tumors after administration to humans (i.e., tumorigenicity risk). Thus, the quality control of the medicinal product is of paramount importance. Often, in particular in treatment of neurological diseases, it is not clear whether a progenitor, precursor, or fully mature cell should be administered. Careful preclinical work is required in each clinical indication to establish the most effective approach. Where the strategy is designed to replace a cell that is lost in a particular disease, the environment into which the cell-based medicinal product is placed may not be supportive of cell survival and integration/persistence. In general, one needs to pay attention to provide a protective environment for the medicinal product.

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