1 Therapeutic stem cells answer a strategic breakthrough need of healthcare

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Introduction

The *Tractatus de Herbis* (Anonymous, 1440) is one of the earliest dictionaries ever written to provide the names and pictures of 'simples', that is, the medicinal plants used during the Middle Ages in everyday therapeutic practice (Riddle, 1974). From this plant-based approach to treating human and animal ailments, the pharmaceutical industry has developed through a process that first aimed at isolating active pharmaceutical principles from extracts. The most telling examples here are perhaps the case of licorice roots (Figure 1.1), reportedly efficacious in curing a number of diseases from the common cold to liver diseases, that has been used in Europe since pre-historic times (Fiorea *et al.*, 2005), or more recently the bark of the cinchona tree that contains quinine, and in Europe that of the willow tree that contains salicin, and the development of aspirin as a modern analgesic drug prepared as pure acetylsalicylic acid, produced on an industrial scale and marketed for the first time in 1899 by the German firm of Friedrich Bayer & Co (Elberfeld, Germany) (now Bayer AG, Leverkusen, Germany) (Tainter, 1948; Sneader, 2000; Brune and Hinz, 2004; Lukovic *et al.*, 2014). Despite having decreased in importance due to the deployment of high throughput techniques to identify and optimise small molecules that act upon targets of well-defined mechanisms of action, natural products still remain a source of important drugs as recently exemplified by the discovery in 1966 of taxol, a compound produced by endophytic fungi in the bark of the Pacific yew tree (Nicolaou *et al.*, 1994). Notably, ethnobotanic medicine, which encompasses the healing traditions of populations worldwide, remains to this day relevant in drug discovery (Fabricant and Farnsworth, 2001). In the foundational years of the modern pharmaceutical industry, pure chemicals were soon being produced by chemical synthesis as a necessity, given the difficulty in procuring the biological raw materials from the Orient and South America, particularly triggered by the blockade of the Continent during the Napoleonic Wars (Crouzet, 1964), to produce drugs such as quinine and morphine (Brune and Hinz, 2004). This first transformation was facilitated by earlier developments in chemistry achieved particularly for the production of dyes along the Rhine in the cities of Basel, Frankfurt, and Köln, which served as the cradle of the modern pharmaceutical industry through a combination of critical success factors comprising skilled workers, a plentiful water resource and easy transportation at the crossroads of several countries representing distinct markets (ibid.).The rise and improvements in ancillary technologies and sciences, such as pharmacology, molecular biology, cell biology, microbiology, human genetics, robotics, as well as bioinformatics have further paved the way for the development of drugs of increasing safety and efficacy to treat an array of indications of increasing complexity. These advances have promoted the emergence and maturation of several technological platforms to develop novel pharmaceutical modalities (Figure 1.2).

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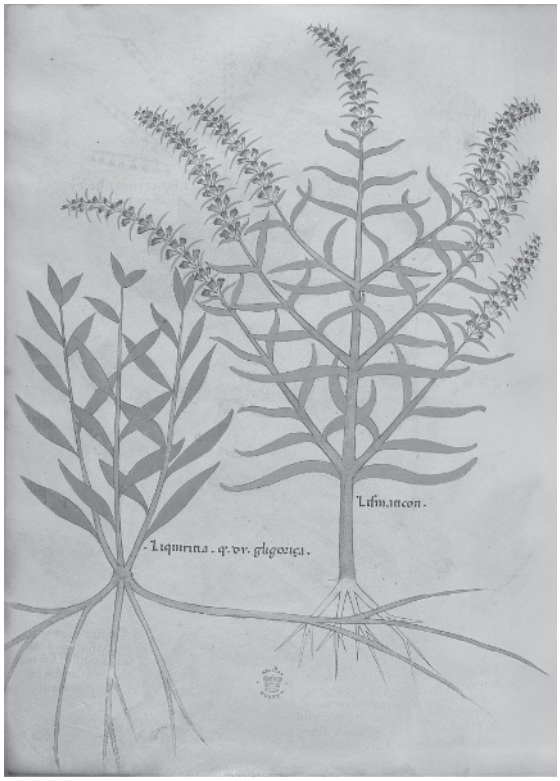


Figure 1.1 Liquorice. From folio f. 53v of the *Tractatus de Herbis* © M. Moleiro Editor (www.moleiro.com). Reproduced with permission (see plate section for color representation of this figure).

Strategic breakthrough need

The greatest challenge in medicine is to develop drugs with positive risk vs. clinical benefits ratios and to understand the bases of adverse reactions to drugs. The first biotechnological embodiment of the properties of stem cells was to enable the development of safer drugs using: (1) hepatocytes and cardiomyocytes to unravel toxicities of compounds in development earlier in the discovery process; (2) cells derived from iPS cells sourced from patients to better reproduce the biology of diseases; and (3) mini-organs, generated, for example, by bioprinting technologies, to enable testing compounds in development on a chip or under the native three-dimensional architectures of organs (Mironov *et al.*, 2003; Nishikawa, Goldstein and Nierras, 2008; Jensen, Hyllner and Bjorquist, 2009; Baker, 2011; Wobus and Löser, 2011). These technologies are already being used in the research laboratories of academic or industrial laboratories (Vertès, 2010). On the other hand, one of the recent developments of this renewed strategic focus of the pharmaceutical industry is encompassed in the concept of personalised medicine, which aims to provide the right treatment to the right patient at the right time, so as to maximise efficacy while minimising adverse side effects and optimising the economic aspects of healthcare (Hamburg and Collins, 2010; Towse and Garrison, 2013). This challenge constitutes the strategic breakthrough need that must be addressed in the coming decade.

The 'magic bullet' concept revisited

This need to develop personalised and tailored drugs that maximise efficacy and reduce side effects by precisely targeting specific infectious organisms or molecular defects but not the host tissue, for example, in a cancer patient, was first advocated by Paul Ehrlich (Winau, Westphal and Winau, 2004; Strebhardt and Ullrich, 2008). These 'magic bullets' would comprise essentially two functional elements: the first functional group would recognise and bind to its targets, while the second would provide the therapeutic action. Immunotoxins have been notably developed using this basic architecture (Brodsky, 1988; Torchilin, 2000).

The pharmaceutical industry of today relies on several technological platforms, with the technology of small molecules having the longest tradition of use. Biologics, therapeutic proteins comprising enzymes and most importantly monoclonal antibodies (mAbs), represent a class of pharmaceuticals that has gained a strong foothold in the market since the beginning of the genetic engineering era in the early 1980s, a technological deployment that has accelerated in the late 1990s to take its full place in the pharmacopeia in the mid-2000s (Galambos and Sturchio, 1998).

Inventing treatments of the future is a complex process. The first step is to define the ideal target product profile that the novel drug needs to exhibit, comprising elements related to reduced toxicity, increased efficacy, or easier delivery as compared to

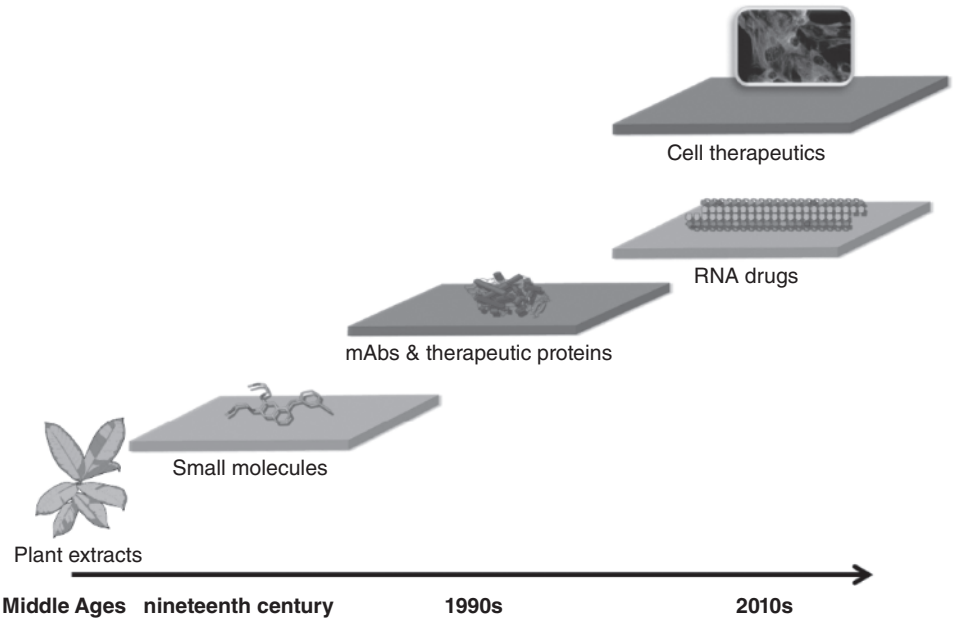


Figure 1.2 Platform technologies that have supported the development of pharmaceuticals throughout the ages. The pharmaceutical industry is deeply rooted in chemistry; however, novel technological platforms have emerged in recent years that have enabled medical practitioners to treat diseases which remained largely intractable using small molecules. In particular, the technology of monoclonal antibodies (mAbs) has revolutionised healthcare since the commercialisation of the first molecule of this class in the late 1990s (Brodsky, 1988; Pescovitz, 2006; Nelson *et al.*, 2010; *Buss et al.*, 2012). Other biotechnological products such as therapeutic proteins are now also part of the pharmacopeia (Pavlou and Reichert, 2004). Nucleic acids drugs (e.g. siRNAs, miRNAs, RNA aptamers, antisense oligonucleotides) and cell therapeutics (e.g., mesenchymal stem cells, hematopoietic stem cells, pluripotent stem cell-derived cells and tissues, tissue-specific stem cells, T-cells and engineered T-cells as well as NK cells) constitute novel pharmaceutical modalities that should come of age starting in the 2010 decade (Opalinska and Gewirtz, 2002; Pecot *et al.*, 2011; Daley, 2012).

the standard of care. Target product profiles can be very specific and with well-quantified thresholds. Notably, the standard of care is typically a moving target, and this dynamics needs to be forecasted early in the process when designing clinical trials and particularly when selecting endpoints, since the new drug could become obsolete even before it reaches the market. An example here is the autologous cytotherapeutic Provenge, the sales of which, shortly after its launch, were directly challenged by Johnson & Johnson's oral treatment Zytiga (abiraterone acetate) as a new first-line treatment in metastatic castration-resistant prostate cancer (Gardner, Elzey and Hahn, 2012; Staton, 2013). Taking the example of designing an appropriate target product profile to develop a novel treatment for Crohn's Disease (CD), a gastrointestinal indication for which mesenchymal stem cells (MSCs) could prove useful (Voswinkel *et al.*, 2013), the major need is to achieve improved CD maintenance therapies, given, on the one hand, the safety risks associated with existing biologics therapies, and, on the other, the tendency exhibited over time by certain patients to stop responding to these therapies, a tendency that leads to inevitable relapses. Furthermore, gastroenterologists indicated in 2009 that, if the emerging product is to secure a price premium of 50% over the price of adalimumab, a leading monoclonal antibody (mAb) CD therapeutic agent, the attribute that influences CD prescription the most is the maintenance of clinical remission, with, for a new product, ideally a novel mechanism of action to treat moderate-to-severe CD patients characterised by placebo-adjusted rates for the maintenance of clinical response, clinical remission, corticosteroid-free clinical remission, and fistula closure that range from 20–30% higher than the

rates observed for adalimumab (Anonymous, 2009). This need was stated as follows: 'The limited number of treatment options that exist for CD patients with steroid-resistant, steroid-dependent, and fistulizing disease offers opportunity for effective therapies that can serve as alternatives' (Voswinkel *et al.*, 2013). Stem cell therapeutics, and particularly MSCs that have anti-inflammatory properties (Bernardo and Fibbe, 2013), constitute paradigm-changing products that respond well to these prerequisites, and thus are worth exploring, including as CD treatments in particular (Voswinkel *et al.*, 2013).

With the recognition that most diseases are heterogeneous in nature and that various biological subgroups can be distinguished, each requiring a specific pharmacological intervention, the conventional paradigm of the 'one disease, one drug, one target', on which the success of blockbusters and the pharmaceutical industry as an asset class has relied, is essentially finished (Jorgensen, 2011). The approach of personalised medicine to understand inter-individual differences in drug responses, including particularly of the genes that predispose patients to adverse drug responses (ADRs) or to varying drug efficacies, is currently used by most pharmaceutical companies (Chan and Ginsburg, 2011; Jorgensen, 2011; Wei, Lee and Chen, 2012). This phenomenon of heterogeneous responses can be exemplified by the subset of high cholesterol patients who fail to respond to statins, or by the large subset of hypertensive patients who fail to respond to β-blockers, despite these molecules providing tremendous clinical benefits to others (Ong *et al.*, 2012). Small molecule- and biologics-based clinical interventions thus need to rely on an approach with more granularity regarding the specific characteristics of each patient, hence they rely on implementing diagnostic tests that enable the practitioner to interrogate a deeper set of well-validated biomarkers to optimally stratify patient populations. Notably, high throughput techniques such as genomics, transcriptomics, proteomics and metabolomics, coupled with nuclear magnetic resonance spectroscopy or mass spectrometry, have opened up parallel paths to develop such novel biomarkers (Rifai, Gillette and Carr, 2006; Pontén *et al.*, 2011; Wheelock *et al.*, 2013). As emphasised by Ong *et al.* (2012), '[the] ability to prescribe drugs only to individuals identified as responders would significantly reduce wasted medical costs. Furthermore, by not prescribing drugs to those genetically at risk for ADRs, the costs associated with caring for patients with untoward drug toxicities could be eliminated.' Notably, ADRs account for 6.7% of all hospitalisations; they comprise the fourth to the sixth most common causes of in-patient deaths in Western countries; and 15% of all ADRs are idiosyncratic reactions for which no dose dependency could be observed (Lazarou, Pomeranz and Corey, 1998; Pirmohamed and Park, 2001; Pirmohamed *et al.*, 2002; Severino and Del Zompo, 2004).

Personalised medicines fully fit within the 'novel rules of 5', empirically determined by Astra Zeneca (Cambridge, UK) following a comprehensive longitudinal study of small molecule projects, whereby the ideal portfolio development model relies on a five-dimensional framework circumscribed by: (1) the right target; (2) the right tissue; (3) the right safety; (4) the right patients; and (5) the right commercial potential (Cook *et al.*, 2014). Adaptive medicines could be defined as a subset of personalised medicine; that is, pharmaceuticals that can adapt to the idiosyncrasies of a particular patient to minimise side effects and maximise efficacy (Figure 1.3a). Adaptive medicines can be mapped according to four ideal fundamental axes (Figure 1.3b): (1) they are characterised by a large safety margin; (2) they have similar effects in a large range of doses; (3) they are activated only in the diseased areas of the body; and (4) they can be manufactured and distributed in a similar manner as a biologics. These four attribute axes define a space of pharmaceutical entities that are underlined by one biological dimension, that is, sensing and responding properties, and one industrial dimension, that is, robustness and industrialisation attributes (Figure 1.3b). The strategic breakthrough need here is to invent, design and enhance the technology platforms that will enable researchers and clinical developers to bring to the market the pharmaceutical products of the future, corresponding to optima of the space of pharmaceutical modalities defined by

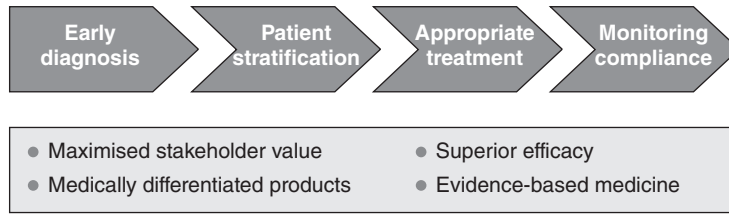


Figure 1.3a Optimising healthcare. Personalised medicine constitutes a new step in the improvement of healthcare. Ideally, a therapeutic product with optimal safety and efficacy attributes will be identified to fit the clinical needs of a particular patient. Such patient stratification can be achieved using companion diagnostics based on well-validated biomarkers. Reduced incidence of adverse events and side-effects is also likely to generate increased compliance. Medically differentiated products with superior efficacies and rooted in evidence-based medicine can lead to maximising the shareholder value of pharmaceutical and biotechnology companies developing personalised drugs as, despite the market for each drug shrinking compared to a one-size-fits-all blockbuster approach, it better responds to the needs of the patients, the prescribers, and the payers; as a result, higher pricing and higher adoption rates can overcome smaller market sizes and particularly so in life-threatening conditions (Gregson *et al*., 2005; Trusheim *et al*., 2007).

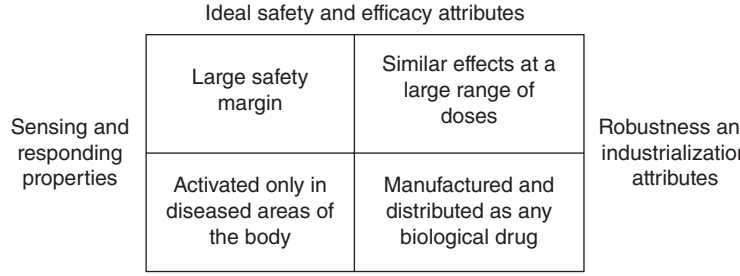


Figure 1.3b Adaptive medicine. Ideal safety and efficacy attributes of pharmaceutical modalities include the capacity of a medicine to adapt to the microenvironment that it encounters in the patient such as to minimise potential side-effects and maximise clinical efficacy. Bacteria sense and respond to their local microenvironments. The litmus test here is whether novel medicines can be developed that mimic this fundamentally natural property of living things to optimise molecular responses to disease environments. Ideally, the new drug's robustness comprises industrial robustness, indicating that it can be reproducibly manufactured on the industrial scale, and clinical robustness, indicating that its safety and efficacy effects are similar at a large range of doses. Allogeneic mesenchymal stem cells appear to have the potential to deliver these characteristics in at least one therapeutic area: the inflammation disease area (Anonymous, 2009; Bernardo and Fibbe, 2013).

these four axes and two dimensions, and congruent with market opportunities that appropriately incentivise and reward financial investments in research and development.

Microorganisms constitute here an interesting proxy to consider. For example, in the prokaryotes, the phosphotransferase system (PTS) has evolved as a complex protein kinase system to enable bacteria to sense the carbohydrate substrates present in their environment and conduct the corresponding molecular signals, transport these sugars intracellularly, and metabolise them while minimising the associated energetic expenses. Remarkably, bacterial PTSs not only mediate the sensing, signalling and transporting of sugars, but also regulate a wide variety of metabolic processes and control the expression of a large array of genes (Saier and Reizer, 1994). In vertebrates, protein phosphorylation regulates most aspects of a cell's life, and, as such, kinases have constituted a very attractive class of drug targets (Cohen, 2002). The ability to sense and respond to the external environment is one of the fundamental capabilities of living things. It is this intrinsic property that provides the underlying basis to achieve the fundamentals of adaptive medicine, that is, where a pharmaceutical modality may have a large safety margin, have similar effects in a large range of doses, and be 'activated' only in diseased

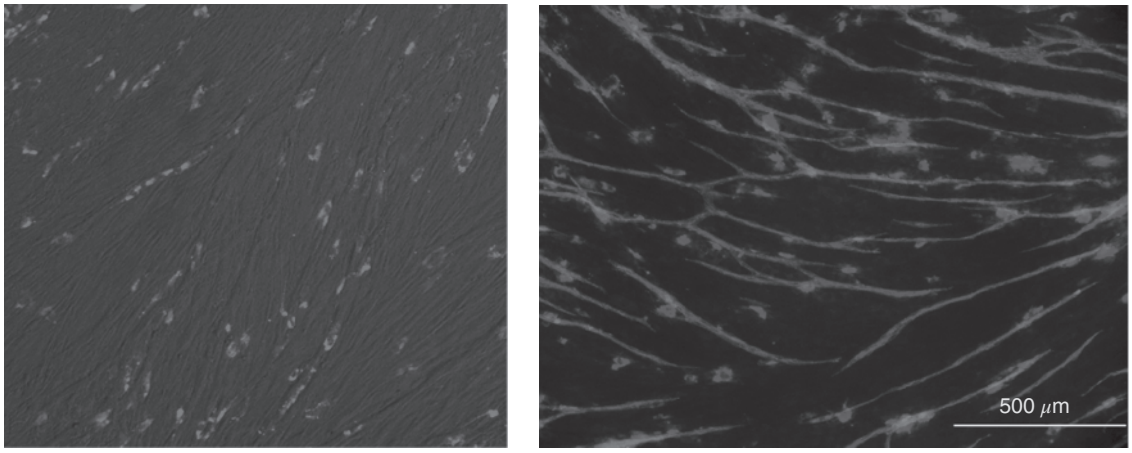


Figure 1.4 Mesenchymal stem cells. Left panel: A dense lawn of human papillary dermal fibroblasts was seeded with unlabelled human umbilical cord vein endothelial cell (HUVECs) on day 0. On day 5, MSCs labelled with the fluorescent dye CM-DiI were seeded and photos of live cultures were taken four days later on day 9. The fluorescent images were taken using a phase contrast objective. Tube-like vascular structures are visible as are the DiI-labelled, MSCs. Notably, only the MSC perinuclear region is labeled. The dark hole in the centre is the location of the nucleus. Right panel: the same culture was fixed with 60% acetone and immune-stained using CD31 antibody (fluorescence) (CD31 is a type I transmembrane protein that is present on an array of cells comprising myeloid cells, platelets, endothelial cells, NK-cells, monocytesand certain $CD4^+$ T-cells). Red and green fluorescent images of the same field were taken and merged. The CD31 immuno-staining confirms that MSCs functionally interact with vascular structures (Sorrell *et al.*, 2009). Credit: photos provided, courtesy of J. Michael Sorrell, Case Western Reserve University (see plate section for color representation of this figure).

areas of the body (Figure 1.3b). Cytotherapeutics exhibit this foundational property.

The value proposition pursued through the development of stem cell therapeutics as *bona fide* drugs will benefit from millions of years of evolution, whereby the healing power of cells is leveraged. MSCs constitute a telling example here. These cells sense and respond to inflammation as follows. Being perivascular cells, they are present on both arterial and venous vessels (Figure 1.4), that is, they are essentially ubiquitous within the body (Caplan and Correa, 2011). They are liberated upon local vessel damage and in turn become activated MSCs that secrete a cocktail of factors, which possess the property of generating a regenerative environment defined as being anti-apoptotic, anti-scarring, angiogenic and mitotic, with MSCs homing to the site of molecular injury and the paracrine factors they secrete impacting dendritic cells, as well as B- and T-cells comprising regulatory T-cells (Treg cells), T-helper cells and killer cells (Uccelli, Moretta and Pistoia, 2008; Caplan and Correa, 2011; Caplan, 2013). Inflammation has evolved as a localised or systemic response to eliminate pathogens and preserve tissue integrity; it is a response to infection, tissue destruction, or injury (Bernardo and Fibbe, 2013). MSCs exert their protective functions by interacting with both the innate and the adaptive immune systems; in particular, they interact with macrophages (Uccelli *et al.*, 2008; Keating, 2012; Le Blanc and Mougiakakos, 2012; Shi *et al.*, 2012; Bernardo and Fibbe, 2013). This action proceeds through a mechanism mediated by pro-inflammatory cytokines secreted by M1 macrophages, or by activated T-cells thereby recruiting MSCs and triggering the release of paracrine mediator factors that trigger the differentiation of monocytes (M0) into M2 macrophages (Figure 1.5a, Figure 1.5b). M1 and M2 macrophages derive from monocytes that, upon encountering an inflammatory environment, can develop either into M1 macrophages, which stimulate local inflammation through the secretion of pro-inflammatory cytokines such as TNF-α and IFN-γ, or into M2 macrophages, which produce a cocktail of anti-inflammatory cytokines, comprising IL-10, TGF-β1, and, but at lower levels, IL-1, IL-6,

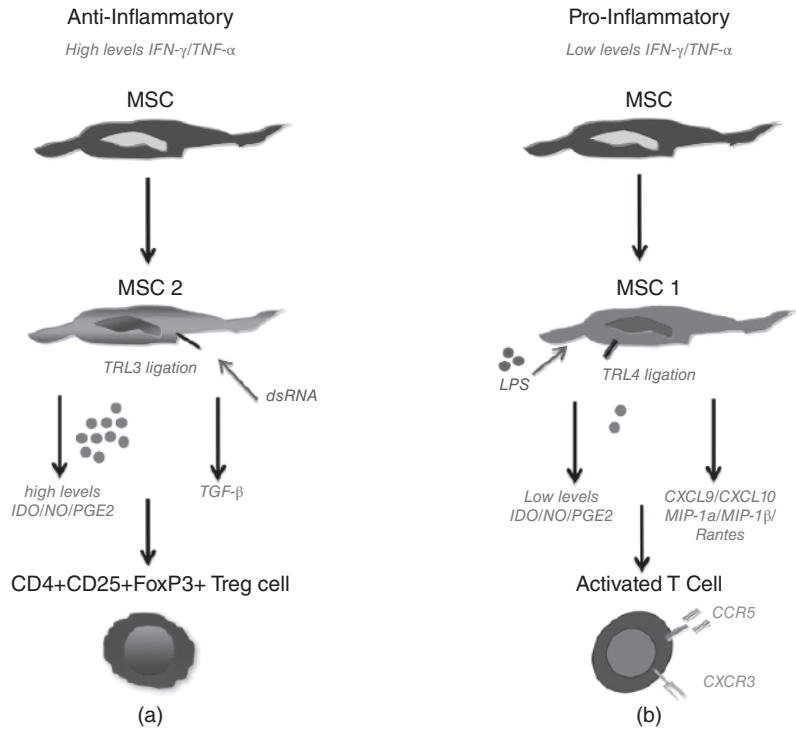


Figure 1.5a Mesenchymal stem cells sense and respond to the inflammatory environment. When subjected to an inflammatory environment (e.g. through high levels of TNF-α and IFN-γ), MSCs become activated and adopt an immune suppressive phenotype, referred to as MSC2, by secreting high levels of soluble factors including indolamine 2,3 dioxygenase (IDO), prostaglandin E2 (PGE2), nitric oxide (NO), TGF-β, hepatocytes growth factor (HGF) and hemoxygenase (HO). Double-stranded RNAs derived from viruses stimulate Toll-like receptors 3 (TLR3) on the MSC surface and may induce polarisation towards the MSC2 phenotype. In parallel with the constitutive secretion of TGF-β by MSCs, this latter phenomenon promotes the emergence of T-reg cells that modulate the immune response. The switch to the pro-inflammatory profile MSC1 is promoted by the absence of an inflammatory environment characterised by low levels of TNF-α and IFN-γ. MSC1 enhances T-cell responses by secreting chemokines, which in turn recruit lymphocytes to sites of inflammation. These chemokines ultimately bind to receptors on the surface of T-cells, such as CCR5 and CXCR3. Moreover, the polarisation towards the MSC1 phenotype can be influenced by the activation of Toll-like receptors 4 (TLR4) by low levels of lipopolysaccharides (LPS) derived from Gram(−) bacteria. TLR ligation triggers phagocytosis and the release of inflammatory mediators that may initiate an innate immune response through macrophages and neutrophils as a first line of defence. Cited and reproduced with permission (Bernardo and Fibbe, 2013) (see plate section for colour representation of this figure).

TNF-α, IFN-γ, as well as TNF-stimulated gene 6 (TSG-6) (Mantovani, 2012; Bernardo and Fibbe, 2013;). This feedback system that balances the phenomenon of M1/M2 macrophage polarisation thus makes MSCs active actors and regulators of the early phases of inflammation, and contributes to maintaining the host's defences while preventing excessive tissue damage that would result from inflammation gone awry (Karin, Lawrence and Nizet, 2006; Bernardo and Fibbe, 2013; Prockop, 2013). The balance between anti-inflammatory and pro-inflammatory pathways is thus assured by four basic elements, as follows: (1) the inducers of inflammation, including microbial, viral and tissue degradation products; (2) the sensors of molecular injury that are constituted by M1 macrophages and mast cells; (3) the mediators that include various cytokines and chemokines; and (4) the effectors that are tissue cells of various types (Prockop, 2013). MSCs, as inflammation sensors, when encountering inflammatory molecules such as TNF-α, become activated, or recruited, and secrete, among other

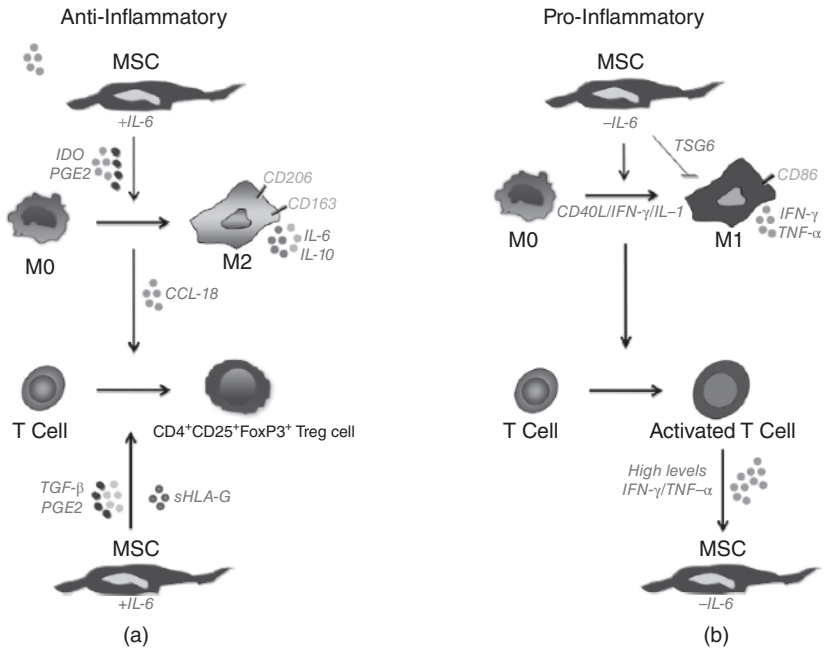


Figure 1.5b Mesenchymal stem cells balance the polarisation of monocytes toward M1 and M2 macrophages. MSCs constitutively secrete IL-6, a cytokine that polarises monocytes (M0) toward M2 macrophages that secrete the anti-inflammatory cytokine IL-10. This polarisation event is dependent on cell–cell contact mechanisms, on the one hand, and on the secretion of soluble factors such as IDO and PGE2, on the other. The polarising effect of MSCs on M2 macrophages is linked to their ability to promote the emergence of $CD4^+CD25^+FoxP3^+$ T-reg cells; which is directly supported by the production of TGF-β by MSCs, and indirectly by the secretion by MSC-induced M2 macrophages that secrete CCL18. Other molecules involved in T-reg generation include PGE2 and soluble HLA-G (sHLA-G). However, in the absence of IL-6, MSCs promote the polarisation of M0 toward pro-inflammatory M1 macrophages; this is mediated by the secretion of IFN-γ and IL-1 as well as by the surface expression of CD40L. In turn, M1 macrophages secrete TNF-α and IFN-γ and express on their surfaces co-stimulatory molecules that promote the activation of T-cells. Interestingly, in a peritonitis model, it was observed that the infusion of MSCs results in the secretion of TSG-6, a molecule that attenuates the activation of peritoneal macrophages, and that the therapeutic effect is mediated by endocrine rather than paracrine mechanisms, thus suggesting that homing to the site of injury is not necessarily required for therapeutic efficacy (Bernardo and Fibbe, 2013). Cited and reproduced with permission (Bernardo and Fibbe, 2013) (see plate section for colour representation of this figure).

molecules, TSG-6, which negatively regulates the pro-inflammatory M1 macrophages, and PGE2, which promotes the development of monocytes into the anti-inflammatory M2 macrophages.

Regenerative medicine products are defined as products that 'replace or regenerate human cells, tissues or organs, to restore or establish normal functions' (Mason and Dunnill, 2008). Considering that totally novel mechanisms of action are leveraged, either by the engraftment of (pluripotent) stem cell-derived cells, or by the delivery of adult stem cells such as haematopoietic stem cells (HSCs) or MSCs, paradigm-changing and disease-modifying products could be developed in all therapeutic areas. With a focus on the six primary therapeutic areas researched by large pharmaceutical firms (Figure 1.6), regenerative medicine can be applied to seek treatments to meet high unmet needs where conventional therapeutics have all but failed. Acute indications such as graft-versus-host-disease (GvHD) constitute areas of particular interest for the development of such emerging medicines, and especially in treating no-hope patients who are refractory to conventional treatments. It is this approach that was followed by Osiris Therapeutics (Columbia, MD, USA), one of the first companies to develop MSCs as drugs, achieving the conditional approval in Canada in 2012 of remestemcel-L (brand name:

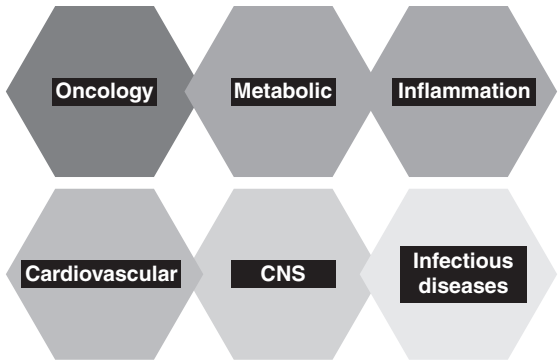


Figure 1.6 The six primary therapeutic areas of large pharmaceutical firms. Data were compiled from the annual reports of global pharmaceutical companies.

Prochymal), an allogeneic MSC preparation (Prasad *et al.*, 2011; Syed and Evans, 2013; Kurtzberg *et al.*, 2014). The treatment of chronic diseases remains challenging, considering existing standards of care and the greater challenges to achieve clear-cut endpoints, as compared to the clearer read-outs of clinical trials in acute diseases. On the other hand, and driven by the fundamental mechanisms of action of stem cell therapeutics, there are areas of opportunities that could be exploited to develop breakthrough drugs. For example, a number of high morbidity chronic diseases are still at present poorly addressed, at least in the long run of the disease. Atherosclerosis, type 2 diabetes, inflammatory bowel diseases (ulcerative colitis and Crohn's disease), as well as Alzheimer's disease, are all examples of chronic diseases pathophysiologically due to an inflammatory component, despite their precise molecular bases and inflammatory stimuli remaining unknown and, if known, being very challenging to modulate (Granlund *et al.*, 2013; Tabas and Glass, 2013). Notably, there are limitations to therapeutically targeting the inflammatory response, albeit some success with anti-inflammatory therapy in chronic diseases has been achieved in certain diseases triggered by primary inflammation dysregulation or autoimmunity (Tabas and Glass, 2013). Given that inflammatory responses are necessary for survival, breakthrough clinical benefits could be achieved with pharmaceutical modalities that optimally adapt to the molecular environment they encounter; here again, stem cell therapeutics such as MSCs have a potential worth exploring. This is exemplified particularly well by the clinical translation of MSC preparations in inflammatory bowel diseases (Van Deen, Oikonomopoulos and Hommes, 2013; Voswinkel *et al.*, 2013; Gazouli, Roubelakis and Theodoropoulos, 2014), or in diabetes and in its complications such as diabetic nephropathies (Volarevic, Lako and Stojkovic, 2013; D'Addio *et al.*, 2014). Pluripotent stem cell-derived cytotherapies also offer treatment options for chronic diseases, as exemplified by the development of encapsulated human iPS-derived or ESC-derived β-cells to serve as artificial pancreas (Calafiore, Montanucci and Basta, 2014; Orlando *et al.*, 2014). Similarly, dry age-related macular degeneration (dry AMD) constitutes an indication where iPS-derived or hESC-derived retinal pigment epithelium (RPE) cells could be deployed, considering the immune-privilege status of the eye, the accessibility of the organ, the ease of the read-out, and the high co-morbidity associated with this disease without satisfactory conventional treatment to this date (Evans and Syed, 2013; Melville *et al.*, 2013; Ramsden *et al.*, 2013).

Reasons to believe in the clinical potential of stem cell therapeutics

As with the development for commercialisation of any breakthrough or game-changing innovation, regenerative medicine, including its segment of cell therapy, faces an uncertain future. The ability of an established company to invest in radical innovation projects directly depends on its willingness to trade off with conventional investments in technologies serving its established markets (Hamel and Prahalad, 1991; Herrmann, Tiomczak and Befurt, 1998; O'Connor and McDermott, 2004). The human side of radical innovation is a key ingredient of success here, and it has been reported that radical innovation projects and investment decisions would optimally be performed by individuals 'who have performed the task over and over to leverage the intuition they gain as a result of rare, infrequent experience' in a critical strategic capability that is built over time (O'Connor and McDermott, 2004). Discounted cash flow (DCF) valuations are financial tools that are

useful to value projects where R&D outlays and pay-offs, as well as project risk, can be estimated relatively accurately using appropriate comparables and sensitivity analyses; however, this traditional investment decision tool falls short in the case of game-changing innovation projects (Remer, Ang and Baden-Fuller, 2001; Christensen, Kaufman and Shih, 2008). Indeed, radical innovation projects are typically characterised by high project risk, particularly technology and market risks, and thus high volatility, which is determined not only by known unknowns, but also by unknown unknowns (Smith, Merna and Jobling, 2013). Real options constitute financial tools that intrinsically express such volatility in potential pay-offs, thereby reflecting the financial asymmetry between the downside risk, which is limited to the cost of purchasing the option, and the upside potential, which remains very large and linked to the value of the underlying asset (Remer, Ang and Baden-Fuller, 2001; Day, Schoemaker and Gunther, 2004; Christensen, Kaufman and Shih, 2008). As such, real option methodologies lead to superior decision-making hints. Remarkably, real option reasoning, rather than calculating real option values, is sufficient in most cases for strategic decision-making, which, as emphasised by Leslie and Michaels (1997), is achieved by increasing the value of option-like projects through a dynamic and flexible process that enables changes to reflect variables in the radical innovation projects that are considered as well as changes in their drivers (Luehrman, 1998; Remer, Ang and Baden-Fuller, 2001). Notably, here, corporate cultural agility constitutes a critical success factor to expand beyond the boundary knowledge of the firm, for example, to access real options to test the fundamentals of a radical innovation project, or to expand the dimensions of a radical innovation project.

Among the assumptions that can guide the valuation of real options in regenerative medicine in general, and in cell therapy in particular, are the following propositions. These represent either intuitive or demonstrated fundamental reasons to believe that cell therapy real options are 'in the money', that is, that the values of their underlying assets exceed the prices of these options, and thus that these are worthy of development.

- *Cell therapeutics are not passing fads, they will transform medicine; the only question is 'How soon?'* This intuitive proposition is supported by parallels with the transformational power that the technology of monoclonal antibodies has had in medicine since their coming of age in the late 1990s (cf. Chapter 33 of the present volume) (Nelson, Dhimolea and Reichert, 2010; Buss *et al.*, 2012).
- *Cells are not only transplants: they can be drugs.* Bone marrow transplantation, a surgery that aims to deliver haematopoietic stem cells, has a long history of clinical use (Thomas, 1999; Santos, 2009;). de la Morena and Gatti, 2011). The adult allogeneic MSC preparation Prochymal has been conditionally approved in Canada for the treatment of monoclonal antibody refractory pediatric acute GvHD (Prasad *et al.*, 2011; Kurtzberg *et al.*, 2014).
- *Cell therapy's first paradigm-changing application is in treating inflammation and autoimmune disease.* Cytotherapeutics deliver clinical benefits that can address medical needs that until now could not be addressed using conventional pharmaceutical modalities. Clinical trials of adult allogeneic MSCs have yielded signals of efficacy in various inflammatory diseases, including particularly refractory GvHD, inflammatory bowel diseases, or osteoarthritis (Davatchi *et al.*, 2011; Prasad *et al.*, 2011; Ricart, 2012; Diekman and Guilak 2013; Nair and Saxena, 2013; Kurtzberg *et al.*, 2014).
- *It is possible to protect the intellectual property of these new drugs.* Numerous patents have already been granted for a variety of therapeutic stem cell products, though embryonic stem cells are not patentable in every jurisdiction, as is the case, for example, of the European Patent Office (EPO) for which such claims cannot be granted on moral grounds (Bergman and Graff, 2007; Nichogiannopoulou, 2011; Elliott and Konski, 2013; Konski, 2013; Nair and Saxena, 2013; Noonan, 2014). Nonetheless, the EPO would grant patents for products derived from embryonic stem cells that have been obtained without the destruction of an embryo (Vertès, 2015).
- *These medicines offer the potential for superior efficacy and disease-modifying benefits with significantly reduced side-effects.* This intuitive proposition, which is the

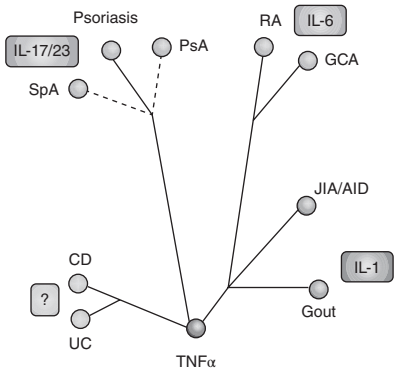
foundation of cytotherapy, is supported by the sensing and responding capabilities of cells to adapt their responses to the environment they encounter. This is exemplified by the paracrine effects of MSCs (illustrated in Figure 1.5) to which therapeutic effects observed in small and large animal models as well as in clinical trials have been ascribed (Meirelles *et al*., 2009; Caplan and Correa, 2011; Bernardo and Fibbe, 2013).

- *It is possible to consistently and economically manufacture these new therapies and maintain their intrinsic attributes throughout the distribution chain.* MSCs can be reproducibly expanded *ex vivo* either on plates (2-D) or in bioreactors (3-D) while tethered on microcarriers, following good manufacturing practices (GMP) and robust process control as well as change control procedures. These advances in manufacturing, including positive selection methods, making use either of cell surface markers or the properties of these cells to adhere to plastic, notably rely on the conventional approach of working in campaign modes using master cell banks and working cells banks. These methods and processes have been key enablers to explore therapeutic uses for these cell populations (Schallmoser *et al*., 2008; Bieback, Kinzebach and Karagianni, 2010; Sensebe, Bourin and Tarte, 2011; Chen, Reuveny and Oh, 2013; da Silva *et al*., 2014; Mendicino *et al*., 2014; Viswanathan *et al*., 2014).

Another lead to consider refers to the indication discovery process. Applying this discovery approach to stem cell therapeutics, it is possible to use in silico discovery tools such as integrative knowledge management to consolidate (Marti-Solano *et al*., 2014), in an open innovation model (Billington and Davidson, 2012), the knowledge that has been generated throughout the discovery process, as well as ontological analyses (Dutkowski *et al*., 2013) and molecular taxonomy trees of diseases (Yang and Rannala, 2012).

Performing an ontological analysis equates to determining the relationships that exist between various entities of a system. A remarkable parallel can be made here again with the field of industrial microbiology where knowledge of bacterial genomics, transcriptomics, metabolomics and fluxomics can be applied to develop novel biotechnological production processes using systems biology tools to predict at steady states reactions, rates, yields or kinetics (Vertès, Inui and Yukawa, 2012). The technology of virtual patients, that is, of in silico models of human biology generated by consolidating knowledge of human molecular biology and enzymology, attained *in vitro* and *in vivo*, has already been put to use in several complex disease areas to model the effect of small molecules drug candidates, as exemplified (among many examples) by work carried out on asthma by Pfizer (New York, USA) and Entelos (San Mateo, CA, USA) (Anonymous, 2003; Rajasethupathy *et al*., 2005). Conceptually, similar models could be generated for novel cell therapeutics also by identifying the cocktail of factors that these cells secrete under specific disease environments. Here again, the paracrine effects of MSCs would represent a very attractive target for this bioinformatics approach by determining which factors are secreted under a variety of conditions, and modelling the impact of these factors on specific diseases by means of systems biology.

Taxonomy trees have proved extremely useful in studying the biology of the living world. Beyond their interest for fundamental research, such trees have proved invaluable for practical applications, such as facilitating the genetic engineering of industrial microbial workhorses (Woese, 1987; Dworkin *et al*., 2006). A similar concept can be deployed to structure the knowledge gained in pharmaceutical research over the years. In this regard, the publicly available data generated using mAbs to drug cytokine targets are particularly worth emphasising to develop stem cell therapies for inflammatory diseases, as these molecules are primary effectors of the immune response. Of note, beyond clinical Phase III and Phase II data, Phase I data also can be used to generate such organised knowledge, since, to progress to the Phase I of development, any compound needs to have convincingly demonstrated efficacy in a relevant pre-clinical model. By compiling such a data set that is rich in data from all the registered clinical trials in a specific indication against a specific target, one can generate molecular taxonomy trees of inflammatory cytokines and the efficacy of cytokine inhibition in chronic inflammatory diseases. An analogy of chronic diseases in human biology is the steady



(a)

CID	TNF	IL-6R	IL-1	IL-12/23	IL-17A
Rheumatoid arthritis					
Giant cell arthritis					
JIA/AID					
Gout					
Crohn's disease					
Ulcerative colitis					
Psoriasis					
Psoriatic arthritis					
Ankylosing spondylitis					
Multiple sclerosis					
Drugs	Adalimumab Certolizumab Etanercept Golimumab Infliximab	Tocilizumab Sarilumab*	Anakinra Canakinumab Rilonacept	Ustakinumab Briakinumab*	Brodalumab* Ixekizumab* Secucinumab*

(b)

Figure 1.7 Molecular taxonomy tree of inflammatory cytokines and efficacy of cytokine inhibition in chronic inflammatory diseases. Left panel: the majority of chronic inflammatory diseases responds to TNF-α inhibition but differ in their responses to the inhibition of other inflammatory cytokines, including IL-6, IL-1, IL-17, and IL-23. These differences suggest a hierarchical structure of cytokine effects in various chronic inflammatory diseases that can be represented as a taxonomic tree. Right panel: dark green indicates strong clinical efficacy of inhibition of each cytokine that was confirmed in randomised clinical trials of various chronic inflammatory diseases; red indicates disease-aggravating effects; grey represents no or mild clinical efficacy, or the absence of relevant data; dashed blue squares represent cytokine inhibition of similar cytokine dependence. IL12/23 represents the combined inhibition of IL-12 and IL-23; IL-6R: IL-6 receptor; RA: rheumatoid arthritis; JIA: juvenile idiopathic arthritis; AID: autoinflammatory disease; CD: Crohn's Disease; UC: ulcerative colitis; PsA: psoriatic arthritis; SpA: spondyloarthritis; GCA: giant cell arteritis. Asterisks indicate drugs that have not been approved by the time of publication of the original paper (2013). Cited and reproduced with permission (Schett *et al.*, 2013) (see plate section for colour representation of this figure).

state of microbial populations, and thus numerous bridges exist between the systems biology of human and microbial research, not only in technical terms but also in terms of biotechnological significance. These trees can be used to infer indications where novel stem cell treatments could prove safe and efficacious, based on a mechanism of action rationale validated by experiments sourced from open innovation (Figure 1.7). This approach is akin to using what has been referred to elsewhere as 'shared molecular etiology', that is, molecular mechanisms of action that are shared across several diseases (Brooks, Tagle and Groft, 2014).

Cytotherapeutics

The first live cell therapeutics were vaccines, stemming from the experiments of Edward Jenner and Louis Pasteur to overcome the controversies of their contemporaries regarding immunisation (Jenner, 1801; Bucchi, 1997). Cellular and viral vaccines initially were live attenuated strains; one of the advantages of using live strains is that they can spread naturally and thus can protect against infection a population beyond those who have initially been inoculated with the vaccine (Plotkin and Plotkin, 2011). For many of these prevention products, maintaining an appropriate cold chain from the point of production to the point of care is essential (Zaffran *et al.*, 2013). Critical learning regarding the appropriate management of the logistics and supply chain of perishable products, such as stem cell therapeutics, can also be derived from the experience gained through decades of collection, processing, quality analysis, transport, storage and delivery at point-of-care of blood products, for which superior performance can be achieved through simple management procedures implemented by experienced staff (Stanger *et al.*, 2012).

Tissue engineering: the example trachea reconstruction

Tissue engineering constitutes for the general public one of the most visible embodiments of the

potential of regenerative medicine. Reconstructing three-dimensional functional organs with normal function (but not necessarily normal shape) is a dream that is becoming a reality; this capability enables the practitioner to consider therapeutic intervention options beyond xenogeneic solid organ transplantation, donor organ transplantation, or cadaveric organ transplantation. Achieving appropriate vascularisation, efficient bio-printing and reduced graft-versus-host disease or transplant rejection are the primary enablers of this novel technology to restore or recreate normal function, or normal external appearance in the case of deep wound repair (Berthiaume, Maguire and Yarmush, 2011; Lanza, Langer and Vacanti, 2011; Cui *et al.*, 2012; Yannas, 2013). The successful use of decellularised matrices sourced from cadaveric tissues has paved the way to functional solid organ generation. Here, the example of tracheal reconstruction using autologous tissues and artificial matrices or matrices of cadaveric origin is particularly worth noting (Grunenwald, Moss and Liberman, 2011; Jungebluth *et al.*, 2011; Gonfiotti *et al.*, 2014). The first such transplantation was performed in 2008 and achieved the long-term restoration of normal tracheal function (Gonfiotti *et al.*, 2014). Similarly, Jungebluth *et al.* (2011) reported on the use of a bioartificial nanocomposite to achieve tracheal reconstruction in a patient with recurrent primary cancer of the distal trachea and main bronchi. In this stunning experiment, a bioartificial nanocomposite was seeded for 36 hours with autologous bone marrow mononuclear cells via a bioreactor. After performing a complete tumour resection, the patient's airway was replaced with this artificial but living tissue, which is notably characterised by an extracellular matrix-like coating and proliferating cells, including a $CD105^{+}$ subpopulation in the scaffold after the reseeding and bioreactor process (CD105, or endoglin, is a molecule that performs a key role in angiogenesis, thus indicating that suitable vascularisation is achievable). Post-operative granulocyte colony-stimulating factor filgrastim (10 μg/kg) and epoetin beta (40,000 UI) were given over 14 days. This surgery did not result in any major complications and restored the patient to a tumour-free state five months after transplantation. Remarkably, the bioartificial nanocomposite lined itself with a vascularised neomucosa, and was partly covered by a nearly healthy epithelium. Moreover, enhanced levels of regenerative-associated plasma factors and the mobilisation of peripheral cells that displayed increased MSC phenotype were observed, as well as the up-regulation of epoetin receptors, anti-apoptotic genes and the miR-34 and miR-449 biomarkers. Together, these observations promote the view that stem-cell homing, cell-mediated wound repair, extracellular matrix remodelling and neovascularisation of the graft all took place (Jungebluth *et al.*, 2011). It is worth noting that in addition to the tracheas, chondrocyte implants to achieve cartilage regeneration, as well as corneas and arteries generated using tissue engineering techniques, also have entered clinical trials (Brittberg *et al.*, 2013; Udelsman *et al.*, 2013; Eisenstein, 2014; Griffith and Harkin, 2014; Lee *et al.*, 2014).

As developed by start-up companies such as Organovo (San Diego, CA, USA), several bioprinting platforms are currently being developed for tissue engineering (Fischer, 2013; Ozbolat and Yu, 2013; Ringeisen *et al.*, 2013; Doyle, 2014; Whitaker, 2014). Notably, enabling correct three-dimensional architectures with native cell types and native functionality makes it possible to generate fully human tissues not only for transplantation, but also, as described in the preceding paragraph, for drug discovery. Importantly, and as emphasised earlier, an exact replica of natural organs is typically not required, but rather what is required are replacement organs that carry the necessary functionality since the shape is not a critical parameter. As an example, the building blocks used by the company Organovo, or bioink, are droplets comprising 10^3 to 10^4 cells; these are loaded onto a NovoGen MMX BioPrinter (Fischer, 2013; Vertès, 2014). The bioprinter builds three-dimensional structures layer by layer at a 20 mm precision to create functional human tissues without scaffolds. Key advantages of this technology are that architecturally correct tissues can be produced with native cell types in their proper locations; this means tissue-like density, true three-dimensional positioning within 200 μ in all axes, multiple tissue-specific cell types, spatially controlled cell compartments, and *in vivo*-like tissue

microarchitectures. Importantly, all these parameters are reproducible as the new bioprinting process is compatible with automated fabrication in a format that is easy to handle (Vertès, 2014). Applications for three-dimensional human tissues that recapitulate human biology include particularly in-organ drug discovery: this is a remarkable advance since human three-dimensional tissue blocks and disease models help better bridge the gap between animal models and clinical trials. These tools are applicable to a wide range of areas, including pre-clinical efficacy, optimisation, early clinical predictability, as well as absorption, distribution, metabolism, excretion and toxicology studies (ADME-Tox). Ultimately, these tools, as they better recapitulate human biology, could help reduce the attrition rate in drug discovery (Di Masi, 2014), hence contributing to a significant decrease in the overall cost of drug development. In transplantation, there is a need for tissues for replacement or to repair organ functions. Tissue-engineered organs could help meet the huge demand for solid organs (Hauptman and O'Connor, 1997; Linden, 2009). Notably, tissues from throughout the body have been mimicked, including lung, heart, breast for oncological drug development, blood vessel, bone, peripheral nerve, skeletal muscle and liver. Large firms are already using this technology for R&D in their laboratories, as exemplified by the large pharmaceutical company Roche (Basel, Switzerland), the biotechnology company United Therapeutics (Silver Spring, MD, USA), or the cosmetics company L'Oréal (Clichy, France) (Vertès, 2014).

Remarkably, bioprinting and tissue engineering spillover innovation also influence the materials industry (Mironov *et al.*, 2003). This can be particularly exemplified by the company Modern Meadow (Brooklyn, NY, USA), which has leveraged these advances to generate capabilities in building leather and meat replacement products. The process developed by this company follows the simple concept of replicating the organisation of collagen in natural tissues. To this end, cells sourced from a biopsy, for example, from a cow, are grown, then assembled in sheets. Those sheets are subsequently layered and ultimately combined to generate a leather replacement product. While distinct from natural leather, this new material exhibits novel properties, including mechanical properties such as different elasticity. It can be tanned, dyed and finished as easily as leather. This novel experiment thus paves the way for biofabrication to enter into a new era of manufacturing, while ensuring the sustainability of resources (Mironov *et al.*, 2003).

Skin substitutes

With Carticel, an autologous cultured chondrocytes product developed by Osiris Therapeutics (Columbia, MD, USA) for cartilage repair, biological skin substitutes to treat diabetic foot ulcers and explored as a treatment for severe burns are the first tissue engineering products to have been commercialised, all since the late 1990s (Gentzkow *et al.*, 1996; Eaglstein and Falanga, 1997; Purdue *et al.*, 1997; Smith, 2014). Dermagraft was approved in 2001 by the US Food and Drug Administration (FDA) for the treatment of chronic diabetic foot ulcers and launched by Advanced Tissue Sciences (San Diego, CA, USA), a company that was incorporated in 1987 but went bankrupt in 2009. Dermagraft is manufactured from human fibroblasts seeded onto a bioabsorbable mesh scaffold; the human fibroblasts divide and grow during the manufacturing process, and secrete a variety of substances comprising dermal collagen, matrix proteins and growth factors. Ultimately, a three-dimensional human dermal substitute is generated that contains metabolically active living cells. Dermagraft can be shipped frozen and has a 6-month shelf life (Pham *et al.*, 2007). The product has been marketed by Shire (Dublin, Ireland); at the time of writing, Dermagraft is commercialised by Organogenesis (Canton, MA, USA), which acquired it in 2014 from Shire (Garde, 2014). Organogenesis also markets Apligraf, a skin substitute that was approved by the FDA for the treatment of diabetic foot ulcers and venous leg ulcers; Apligraf is comprised of an allogeneic cell bi-layer: an outer layer of protective human fibroblasts in a bovine type I collagen matrix, and an inner layer of keratinocytes contained within collagen; however, it has a relatively short shelf life of only 10 days (Eaglstein and Falanga, 1997; Curran and Plosker, 2002). Apligraf was the first living, allogeneic, cell-based product

to receive FDA approval; it was approved for the treatment of venous ulcers in 1998 and for the treatment of diabetic foot ulcers in 2000. Novartis (Basel, Switzerland) had acquired global marketing and distribution rights of Apligraf but retransferred those rights back to Organogenesis when the latter filed in 2002 a voluntary petition for reorganisation under Chapter 11 of the US Bankruptcy Code (Anonymous, 2002).

The commercialisation of skin substitute products has thus been chaotic and has notably been greatly affected by a significant reduction in reimbursement (Carroll, 2013; Garde, 2014; Palmer, 2014). These products are nevertheless becoming commodities. Of note, the safety and efficacy of bioengineered skin substitutes compared with biological skin replacements or standard dressing methods in the management of burns were assessed through a systematic review of the literature. A total of 20 randomised controlled trials were reviewed, resulting in the conclusion that the bioengineered skin substitutes Biobrane (a biocomposite dressing made of nylon fibres embedded in silicone to which collagen has been chemically bound; it was first introduced in 1979 for the treatment of burn wounds), TransCyte (composed of newborn fibroblasts that are grown on the nylon mesh of Biobrane; it was approved for sale in the USA by the FDA in 1999), Dermagraft, Apligraf, autologous cultured skin, and allogeneic cultured skin, are all at least as safe as biological skin replacements or topical agents and wound dressings. Regarding partial thickness burns, the bioengineered skin substitutes Biobrane, TransCyte, Dermagraft, and allogeneic cultured skin, are at least as efficacious as topical agents and wound dressings or allograft (Pham *et al*., 2007).

Haematopoietic stem cells and mesenchymal stem cells

The near future in terms of cytotherapies undoubtedly lies with the therapeutic potential of adult stem cells: HSCs and MSCs. HSC transplantation has become the standard of care for patients with defined congenital or acquired disorders of the haematopoietic system or with chemosensitive, radiosensitive, or immunosensitive malignancies: in 2006, a total of 50,417 first HSC transplantations were performed worldwide, 43% of which used allogeneic HSCs and 57% used autologous HSCs (Gratwohl *et al*., 2010). Notably, bone marrow grafts are supplemented as a stem cell source by HSCs derived from peripheral blood or cord blood, with more than 14 million typed volunteer donors or cord blood units from the many registries worldwide providing stem cells for patients without family donors (Gratwohl *et al*., 2010). On the other hand, as the number of cells is the major limitation of umbilical cord blood transplantation, which can lead to increased risks of graft failure, delayed haematological recovery as well as prolonged immunosuppression, ex vivo stem cell expansion technologies are being developed with the aim of increasing the number of total nucleated cells and $CD34^+$ cells (CD34 is a glycoprotein that is used as a surrogate marker of HSCs and progenitor cells) from single cord blood units, and thus improving clinical outcomes of cord blood transplantation (Berenson *et al*., 1991; Norkin, Lazarus and Wingard, 2013; Beksac and Yurdakul, 2014). For example, Gamida Cell (Jerusalem, Israel) is using a copper chelator technology to generate such grafts from a portion of a single unit of umbilical cord blood that is in turn transplanted in combination with non-expanded cells from the same unit. This product, StemEx, is in clinical trial Phase II/III (de Lima *et al*., 2008; Beksac and Yurdakul, 2014). Various other technologies are being developed to improve the outcome of bone marrow transplantation, including additional cell expansion technologies such as expansion in the presence of nicotinamide, also developed by Gamida Cell as NiCord, or the co-culture of HSCs with MSCs, which was experimented by Mesoblast (Melbourne, Australia) resulting in an expansion of total nucleated cells by a median factor of 12 and of $CD34^+$ cells by a median factor of 30 (de Lima *et al*., 2012; Norkin, Lazarus and Wingard, 2013). A complementary approach aims at improving the engraftment and homing properties of HSCs, for example, by performing an ex vivo enzymatic fucosylation of the extracellular membrane of HSCs, with the rationale of enhancing the native homing and engraftment molecular machinery (Chute, 2006; Taupin, 2010).

MSCs have the property of avoiding immune responses (Ankrum, Ong and Karp, 2014), and thus can be used either allogeneically, that is, sourced from an unrelated donor, or autologously, that is, a biopsy containing MSCs is collected from the patient and these cells are subsequently expanded *ex vivo* prior to being delivered to the same patient for therapeutic purposes. The first MSC products have already reached approval, as exemplified by Cartistem, developed by Medipost (Seoul, South Korea), an allogeneic sodium hyaluronate MSC preparation derived from umbilical cord blood, that was approved in South Korea in 2012 for knee cartilage regeneration; similarly, the adipose tissue-derived autologous stem cell preparation Cupistem, developed by Bukwang Pharmaceutical Co. Ltd. (2014 market capitalisation of approximately $520 million) affiliate's Anterogen Co. Ltd. (Seoul, South Korea), was approved for the treatment of anal fistulas, also in 2012 (Wohn, 2012). Notably, South Korea approved the first stem cell therapeutic in 2011, Hearticellgram-AMI, for the treatment of acute myocardial infarction (Wohn, 2012). This product, developed by FCB-Pharmicell (Seongnam, South Korea) consists of autologous bone marrow-derived MSCs directly injected into the damaged heart. Prochymal is another allogeneic bone marrow-derived MSC preparation, originally developed by Osiris Therapeutics (Columbia, MD, USA) but acquired in 2013 by Mesoblast (Waltz, 2013). Prochymal was conditionally approved in 2012 in Canada and in New Zealand for the treatment of pediatric acute GvHD (Gardner, Elzey and Hahn, 2012; Law, 2014; Newell, Deans and Maziarz, 2014). Importantly, these approvals occurred after Phase III randomised, placebo-controlled trials failed to meet their primary endpoints of durable complete response, but subset analyses demonstrated efficacy in selected patient populations (Newell, Deans and Maziarz, 2014). As a result, still to this date, questions remain regarding the factors that impact the immunomodulatory properties of MSCs, such as their source tissues, *ex vivo* expansion methods, as well as timing and dosage of infusions, and how to optimise these products in order to enhance their clinical efficacies (Goodrich and Hematti, 2014).

The future: pluripotent stem cells-derived cytotherapeutics

Pluripotent stem cells, iPS and hESCs, have an outstanding potential to bring totally novel therapeutic options that are totally unthinkable when using only conventional pharmaceutical modalities. Given their capacity to differentiate into virtually any cell type of the body, these cells are currently being investigated for cellular replacement therapy.

Cytotherapeutics derived from hESCs, such as oligodendrocyte progenitor cells or neural cells, were first tested in a Phase I clinical trial of spinal cord injury by Geron (Menlo Park, CA, USA) to enhance remyelination and promote motor functions; however, despite promising pre-clinical results attained in rodent models of spinal cord injury, Geron halted this trial shortly after its initiation, claiming it did so to better manage corporate business risks (Keirstead *et al.*, 2005; Sahni and Kessler, 2010; Sharp *et al.*, 2010; Nakamura and Okano, 2013; Lukovic *et al.*, 2014). Nevertheless, clinical development for spinal cord injury remains active, as exemplified by Asterias Biotherapeutics (Menlo Park, CA, USA) which acquired the program from Geron, and by StemCells Inc. (Newark, CA, USA) which received approval in December 2010 from the Swiss regulatory agency for therapeutic products, Swissmedic, to initiate a Phase I clinical trial of foetal brain-derived human central nervous system stem cell population (HuCNS-SC cells); the trial is being conducted in Switzerland at the Balgrist University Hospital, University of Zürich.

As alluded to above, a promising embodiment of the technology of pluripotent stem cells is the development of RPE cells derived from hESCs or iPS. Notably, following successful pre-clinical experiments to preserve photoreceptors and preserve or restore visual function, subretinal transplantation procedures of functional RPE cells to replace dysfunctional ones are currently being clinically tested by several companies worldwide, to treat blinding diseases for which there is no appropriate treatment yet, such as dry AMD or Stargardt's macular dystrophy, including Pfizer (New York, USA) in collaboration with the University College London (London, UK), Ocata Therapeutics (previously Advanced Cell Technologies) (Marlborough, MA, USA), the Biotime Inc.

(Alameda, CA, USA) subsidiary CellCure Neurosciences Ltd (Jerusalem, Israel) (CellCure's other notable shareholders include the generics manufacturer Teva Pharmaceutical Industries Ltd (Petach Tikva, Israel) and the technology transfer company of the Hadassah University Hospital in Jerusalem, Hadasit Bio-Holdings Ltd (Jerusalem, Israel)), Dainippon Sumitomo Pharma Co. Ltd (Osaka, Japan) in collaboration with Healios K.K. (Tokyo, Japan) (formerly, Retina Institute Japan K.K.; Healios is a venture from RIKEN (Saitama, Japan), the largest comprehensive research institution in Japan), as well as CHA Bio & Diostech (Seoul, South Korea) (Lu *et al.*, 2009; McKernan, McNeish and Smith, 2010; Bull and Martin, 2011). What is more, other cytotherapeutic approaches to address retinal degeneration are also being explored, exemplified by neural stem cells developed by StemCells Inc., autologous adipose tissue-derived cells developed by Bioheart Inc. (Sunrise, FL, USA), autologous $CD34^+$ bone marrow-derived stem cells developed by the University of California (Davis, CA, USA), bone marrow-derived stem cells for retrobulbar injection developed by the Retinal Associates of South Florida (Margate, FL, USA), or encapsulated human cells genetically modified to secrete ciliary neurotrophic factor (CNTF), which preferentially stimulates and protects neural cells, including, in particular, photoreceptor cells, developed by Neurotech Pharmaceuticals (Cumberland, RI, USA) for a variety of ocular indications comprising, beyond dry AMD, retinitis pigmentosa, macular telangiectasia and achromatopsia. It is also worth noting the autologous cultures of limbal stem cells for the regeneration of destroyed corneal epithelium (brand name Holoclar, conditionally approved by the European Medicines Agency in February 2015) developed by, among many others, Holostem Terapie Avanzate (Modena, Italy), a spin-off of Chiesi Farmaceutici S.p.A (Parma, Italy) and the University of Modena and Reggio Emilia (Modena, Italy) (Pellegrini *et al.*, 2014). Furthermore, the convergence of innovations by distinct business segments may result in products with superior attributes, such as by combining a medical device with a cytotherapeutic, for example, contact lenses with limbal epithelial stem cells to treat limbal stem cell deficiencies, or wound dressings with stem cells as healing enhancers to treat diabetic foot ulcers (Moura *et al.*, 2013; Gore *et al.*, 2014).

The therapeutic potential of pluripotent stem cells can furthermore be illustrated by the generation of encapsulated pluripotent stem cell-derived insulin-secreting β-cells for the treatment of Type 1 diabetes (Godfrey *et al.*, 2012; Holditch, Terzic and Ikeda, 2014; Liew and O'Brien, 2014; Newby, Terada and Mathews, 2014). Remarkably, epigenetic memory mechanisms in key β-cell genes may be in play, suggesting that β-cell-derived iPS may exhibit superior differentiation potential into insulin-producing cells (Bar-Nur *et al.*, 2011). The encapsulation of such β-cells, for example, within a bilaminar polytetrafluoroethylene (PTFE) membrane system, not only prevents immune rejection phenomena, thus alleviating the need for chronic immunosuppression when using allogeneic material, but also sets the therapeutic cells in an implantable and retrievable device (Kirk *et al.*, 2014; Steele *et al.*, 2014).

Many of these techniques are clearly relevant to an array of applications in healthcare, with proof-of-principles having already been achieved pre-clinically for the different functional elements constituting 'replacement living artificial organs or tissues', comprising: (1) sourcing, isolation and manufacture of pluripotent stem cells; (2) differentiation of pluripotent stem cells into cell types of interest; (3) encapsulation of therapeutic cells in an implantable and retrievable device; and (4) delivery of therapeutic cells. There are nevertheless key technical hurdles that remain, including ensuring the avoidance of genetic or epigenetic abnormalities, achieving robust confidence in safety and the development of enhanced differentiation protocols or manufacturing techniques relying on positive isolation procedures, so that the carry-over of residual pluripotent stem cells in the final cytotherapeutic products is avoided, given the teratoma formation potential of undifferentiated pluripotent stem cells; notably, pluripotent cell lines have been observed to vary in their ability to differentiate into desired cell types *in vitro* (Thomson *et al.*, 1998; Fujikawa *et al.*, 2005; Cahan and Daley, 2013; Miura *et al.*, 2014;

Sanchez Alvarado and Yamanaka, 2014; Tabar and Studer, 2014).

Perspectives

Religious or ethical considerations have greatly influenced the type of stem cell research that is being carried out in any particular jurisdiction, and particularly regarding how research on hESCs is considered (El Sheikh and El Sheikh, 2011). Bans on financing the development of hESCs using national funds, or patent policies that deny on moral grounds the granting of claims linked to embryonic stem cells, have likewise influenced research orientations (Levine, Lacy and Hearn, 2013; Noonan, 2014). This has provided a welcome impetus, for example, to trigger the development of the iPS technology in Japan (cf. Chapter 24 of the present volume) (Ishii, Pera and Greely, 2013), or to promote efforts on adult stem cell research, such as on MSCs (Vertès, 2014).

The emerging technology of live stem cell therapeutics constitutes a novel answer to tackle diseases for which conventional products, small molecules and biologics, have all but failed. Given their sensing and responding properties that have been developed through millions of years of natural evolution, live stem cells and their derivatives represent a transformational therapeutic potential, whereby clinical efficacy can be achieved, either by engraftment of these cells, that is, by replacement of dysfunctional cells, or by the addition of normal cells to restore normal function, or by paracrine effects, that is, by the secretion of an array of biological molecules. Remarkably, the pharmaceutical responses provided by live cytotherapeutics are adapted to the environmental cues that these cells encounter locally once delivered to a patient, and hence provide a response that is 'personalised' to the idiosyncrasies of each patient.

The therapeutic space that this novel technology opens has to this date still been virtually untouched, though it is deeply rooted in the already long history of bone marrow transplantation, solid organ transplantation, and even in that of blood products. Exploring this novel space of pharmaceutical intervention and translating in terms of clinical benefits those discoveries are what will mark the coming healthcare decade. As transformational as, in its time, was the transition from using bovine pancreas extracts for treating diabetes to using recombinant insulin produced by genetically modified microorganisms at the hectolitre scale in industrial bioreactors (Lakhtakia, 2013; Pathak, Sinha and Sharma, 2013; White, 2014), live cell therapeutics, functional man-made living replacement organs, or engineered tissues are pharmaceutical modalities that answer a breakthrough need in medicine: achieving, on the one hand, adaptive medicine and, on the other, the commoditisation of man-made living replacement human organs and tissues. What is more, yet unknown intersections with conventional pharmaceutical procedures, with surgical procedures, or with medical devices and combination therapies, for example, for reconstructive surgery to repair very deep wounds, will further expand the scope of those game-changing innovations to enable the successful treatment of diseases or medical conditions that at present are intractable.

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