Assessing the value of autologous and allogeneic cells for regenerative medicine

The advantages and disadvantages of autologous and allogeneic human cells for regenerative medicine are summarized. The comparison of relative advantages includes: ease and cost of treating large numbers of patients, the speed of availability of therapy and the differing complexity of the development pathways. The comparison of relative disadvantages deals with issues such as variability of source material, the risks of cell abnormality and of viral and prion contamination, and the sensitive issues surrounding use of embryo-derived cells. From the comparisons, several potentially decisive issues are drawn out, such as possible immune response and teratoma formation, the impact of patents and the virtues of hospital versus industry-centered development.

KEYWORDS: allogeneic ▪ autologous ▪ bioprocessing ▪ cell therapy ▪ clinical translation ▪ commercialization ▪ regenerative medicine ▪ stem cells ▪ tissue engineering

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The nature of regenerative medicine will be profoundly influenced by whether the cells used are universal (allogeneic) or patient-specific (autologous) [1]. It is unlikely to be wholly one or the other that will dominate, however, at this early stage it is worth examining the issues, both scientific and translational, which favor each and what challenges they bring. Not all are technical and for a period some of the nontechnical issues may be decisive.

Scientific and clinical advances in the last several years have added new dimensions to the consideration of human cell therapies. Most notable has been the capacity to produce induced pluripotent stem (iPS) cells from adult cells. This could have a major impact on both allogeneic and autologous cell therapies. iPS cells may ultimately, although probably not quickly, replace embryonic sources for allogeneic therapies. Equally, they could offer a route to a much wider range of autologous cells for therapy such that even now it is becoming necessary to speak of natural autologous cell therapies (e.g., chondrocytes used in several commercial treatments for damage to the cartilage of the knee) and potential iPS cell-derived autologous therapies. The latter, at present, raise the concern of teratoma formation (benign) and indeed, given the genetic intervention that was used initially, perhaps of other complications too, such as potential malignancy [2]. It is also important to note that the transplantation challenges specific to iPS cells are just starting to be debated and must not be underestimated [3]. In terms of other major changes, the rather ill-defined action of both allogeneic and autologous cells, typified by many attempts to address congestive heart failure using bone marrow-derived cells, are giving way to more concrete evidence of disease-modifying therapies with cells or mixtures of cells. One recent example is that of treatments for immune conditions such as Crohn's disease and graft-versus-host disease. The Phase II clinical trials of allogeneic bone marrow-derived cell therapies for these conditions by Osiris Therapeutics Inc. (Columbia, MD, USA) was a strong demonstration of promise, although the Phase III trial for Crohn's disease has subsequently been discontinued owing to a major design flaw in the clinical trial methodology producing significantly higher than expected placebo response rates [201]. Using similar approaches, efforts to limit normal disease progression in conditions such as stroke and heart attack may be extremely valuable if they can prevent secondary cell damage following the initial event, even if they do not lead to cellular regeneration [4]. Therefore, both allogeneic and autologous cell therapies may come to be subdivided into two types. First, there are those, which by transiently addressing acute conditions, can limit further damage by interrupting the natural disease progression. Second, there are approaches that address chronic conditions where only full regeneration suffices. Although these distinctions are only now beginning to emerge, they need to be borne in mind in what follows where the cited literature mostly predates the distinction. We begin by addressing potential

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advantages and disadvantages of the two sources (Box 1) and then draw out issues that may have a particularly strong influence on the outcome.

Potential advantages of allogeneic cells

For universal (allogeneic) cell material [1] the great virtue is that it is capable of representing a commercial technology more comparable with that of molecular pharmaceuticals than autologous cells (Box 1, allogeneic advantages). This is because it is possible to envisage production by scale-up with product characterization, which defines safety, applied to relatively large batches of material. This has considerable potential economies of scale and more readily allows the

Box 1. Potential advantages and disadvantages of allogeneic and autologous therapeutic cells.

Advantages

- Allogeneic
  - Producing cells for many patients is more efficient
    - Scale-up can go much further
    - Quality control (QC) can be applied to larger lots
    - Existing attachment cell technology for production scale is useful
    - Material of high consistency
    - Allows high patient throughput
  - Cells are always available
    - Can address emergency indications
    - Represents a good commercial opportunity for cell suppliers/contract manufacturing organizations (CMOs)
  - No patient biopsy needed
    - Less clinical time and resources
    - Avoids needing biopsy consent from severely ill patients
  - Commercial product orientated
- Autologous
  - Avoids immune rejection
    - Does not require costly immunosuppression and its associated complications
  - May be easier to proceed with, for example, no requirement for cell line development
    - Reduced start-up costs
    - Avoids embryonic sources
    - Simpler regulations
    - Avoids nondonor virus and prion concerns
    - May avoid cell abnormalities given less expansion for individual patient's requirement
  - Potential for 'point-of-care' processing
    - Could enable independent clinical technology
  - Favored for bioaesthetic applications
  - Service model orientated (e.g., embedded in a hospital or clinic)

Disadvantages

- Allogeneic
  - Immune rejection may be a major issue
  - Risk of cell abnormalities, particularly with many cycles of *in vitro* replication
  - Teratoma formation risk is a concern
  - Provision and consenting of donated cells requires significant time and resources
  - Development investment is high
- Autologous
  - Variability of source material
  - Difficult to generate large numbers of cells from either somatic or adult stem cells
  - Inability to deal with emergencies
  - Patient throughput will be relatively low
  - Difficult to address large numbers of patients at reasonable costs
    - Minimal economies of scale
  - Biopsy procedure is not without risk to patients
  - Any processing failure involves major treatment delays

conventional approach of pharmaceutical quality control. The very demanding analyses for human cells to guarantee quality mean that being able to apply these to large lots is a great gain. Therefore, the potential for large pharmaceutical companies to more readily recognize in allogeneic therapy a process development model with which they are familiar is of major importance. Given the pressing need for treatments, this is a potential advantage that must be heavily emphasized before describing the possibilities of using autologous cells to avoid certain technical or political and ethical challenges of embryo-derived allogeneic cell use.

Like autologous cells, allogeneic ones may also be expanded by scale-out of multiple cultures. This has the advantage of very flexible scaling by simple additions of extra identical culture units. However, if allogeneic cells eventually follow the pattern of production of biopharmaceutical molecules, and particularly vaccines made using attachment cells, then the greater simplicity of scale-up in far fewer production units may prove attractive. As we have noted [5], uniform culture with large areas of attachment cells is not without difficulties and, particularly if patient-sized sheets of allogeneic cells are desired, scale-out may be most convenient.

In medical emergencies, allogeneic cells have several other advantages. First, given reasonable cell stability during storage, there will be an immediately available supply in the clinic. Such stability is not assured and as more sophisticated cells are targeted it may be harder to achieve. For engineered tissue shelf-lives tend to be a matter of days. A further value in nonelective treatments is that these therapies do not require a preliminary biopsy from a potentially severely ill person, thus avoiding an additional procedure and all the associated challenges including appropriate patient biopsy consent. The largest commercial scale therapy at this time, the bilayered dermis/epidermis skin substitute, Apligraf® (Organogenesis, Canton, MA, USA) is an allogeneic cell-based product, which points to the potential for the category.

At present, it is not possible to know when and whether iPS cells can be produced by clinically safe techniques [2,6]. However, intensive research is being conducted to achieve conversion to iPS cells using genes in safer formats, or by employing proteins or small molecules. The subject will be returned to later in addressing the potential advantages of autologous cells where the use of iPS cells to widen the range of therapies is creating much interest. For allogeneic cells perhaps the greatest potential gain of iPS would be not just to avoid the 'pro-life' objections to using embryonic sources, but to provide a more clinically desirable one from the donor perspective. The great majority of human embryonic stem cells (hESCs) have been derived to date from embryos surplus to *in vitro* fertilization (IVF) therapy [7]. However, to increase the supply of therapeutic cells, even with the subsequent substantial cell expansion possible, would require additional sources. There is still much debate over the issue of buying and selling of such material for research and therapies [8]. Condic and Rao note that a poll of the US public in early 2008 showed 47% were in favor of using surplus IVF embryos for research whereas only 18–30% supported creation of embryos solely for research purposes [9]. It is clinically desirable for the reason given below that, if possible, eggs are not taken from women unless they are undergoing IVF procedures or there is some particular humanitarian need. However, it is equally to be hoped that for a period the public recognizes the continuing importance of good availability of hESCs. It is clear that iPS-derived cells are not yet close to representing an alternative and if changes in IVF treatment occur that result in fewer surplus eggs, there may be a serious shortage of hESCs to allow the development of therapeutic procedures. It would be very tragic for potential patients if this happened at just the time when the field is showing great therapeutic promise.

The option of somatic cell nuclear transfer offers a different route where the pluripotent cell is matched to a somatic cell donor [10] but Condic and Rao have noted reservations [9]. Another alternative to embryonic cells, which removes the most obvious objection to using fertilized embryos, is to *in vitro* stimulate unfertilized donor eggs to undergo division (parthenogenesis) to form blastocysts from which pluripotent hESC-like lines can be derived [202]. One potential advantage here is that the cells express just the maternal alloantigens (antigens that are part of the body's self-recognition system) so that deriving a set with potential for immune matching is significantly easier. These cell lines could potentially be used for both autologous (egg donor) and allogeneic therapies [11]. Whilst ethically more acceptable to a larger proportion of the population, the collection of eggs has potential risks, for example ovarian hyperstimulation syndrome, and therefore is not acceptable to all [12].

Potential advantages of autologous cells

A great advantage of natural patient-derived (autologous) cells (**Box 1**) [1] is that there will be no host-versus-graft immunological reaction. Chen

and Palmer suggest potential problems but it will be important to distinguish local inflammatory changes, which are inevitable naturally, but do not pose a threat [13]. Not only do autologous cells avoid the risks of lifelong immunosuppression therapy, but also its significant costs so that the therapy has potentially the full long-term financial gain of a regenerative medicine. If the cells can be readily prepared, autologous therapy is something that clinicians can proceed with themselves, at least to early clinical trials, although they still need to meet regulatory requirements [6]. That almost certainly accounts for the large number of reported trials, especially in conditions such as heart failure where currently clinicians have little else to offer than modest amelioration. For those clinicians wishing to provide more than this for their patients the option of autologous cells allows them to go forward at their hospital rather than having to wait for commercial products where financial returns are hard to see as yet. The approach side-steps the use of hESCs and all the legal and ethical battles that their use can provoke in some countries. The limited potential expansion compared with embryo-derived allogeneic cells may reduce the risk of cell abnormalities arising when cells are replicated many times *in vitro* [14]. In some instances, such as that described later, the autologous source allows biopsy of sufficient cells so that no expansion is required.

Most notably in the case of cells from a patient's adipose tissue, considerable technical input has been made to produce equipment that can harvest and process autologous cells, without expansion, at the points of care [15]. Where such minimal manipulation procedures succeed it will further encourage technology that could be operated by clinicians working independently. For patients, and in countries such as Japan, where there are religious or cultural constraints on donated cadaveric material for tissue or organ replacement, this use of autologous material would plainly be attractive. Lin *et al.* have described the characterization of adipose tissue-derived cells isolated with the Celution™ system of Cytori Therapeutics Inc. (San Diego CA, USA) [16]. They defined a set of markers for the heterogeneous cells and showed the cells were equivalent to those isolated manually. Although such a device eases the route for the clinicians, it bears a regulatory burden and it is consistent with the demands made that a sizeable team of engineers worked on the Celution system in a collaboration with the Olympus Corporation of Japan [203].

Because taking autologous treatments to the clinic involves less cell-line development compared with allogeneic ones, it has been argued that they may be more favored by venture capital funders than those derived from pluripotent cell sources [17]. However, although there are certainly many more startups and public sector activities in the autologous sector, pharmaceutical companies, with rare exceptions, are not at present positive about the prospect of processing individual patient material so the exit route for venture capital-funded companies is unclear. It has proved difficult to achieve a profitable business with first-generation simpler autologous cell therapies such as cartilage replacement. However, successful treatment of major diseases, where there is no presently successful therapy and the cost of care is very high, could represent a more feasible business. It may also be considered a sensible governmental expenditure for a clinical service in which the production of cells is either a public or private sector element. Then cell therapy would become equivalent to say, kidney dialysis, and potentially curative.

In the case of bioaesthetic treatments, such as those for baldness or skin wrinkles, there are indications that people prefer autologous cell treatment and they will be paying directly (**Box 1**, advantages). The attraction of autologous material here is perhaps because the procedure is less driven by medical necessity and use of their own cells by an individual will seem more natural [18]. Certainly the risk-to-benefit ratio for autologous cells is probably more appropriate for these non-medical applications, although it remains necessary to avoid risks such as those which could arise from the processing of the cells such as the use of bovine serum albumin. The issue of prion disease, such as new variant Creutzfeldt–Jakob disease, in a donor of allogeneic cells is one additional reason cited. Statistically this is a highly unlikely event but were it to happen with an allogeneic source it would potentially affect a very large number of recipients.

In terms of potential advantages of autologous cells, the use of iPS cells derived from adult cells as originally conceived [19] could not directly be applied safely to clinical goals [2], but the potential this method is rapidly opening up is exciting. Its special significance for autologous therapies is that it can greatly widen the range of possible specialist cell types that could in principle be made available to match the patient's immune system. The derivation of a personalized iPS cell source would preserve the advantages of lack of immune response and avoidance of nondonor virus and prion concerns but would introduce the

disadvantage of potential tumor formation and would move the therapy away from 'point-of-care' treatment. Efforts to reduce the potential dangers of using viral vectors and of enhancing the effect of a smaller vector set or using small and macromolecules as activators are developing rapidly and have been reviewed by Maherali and Hochedliner [20]. For example, in early 2009, two papers described how iPS cells can be prepared using a single multiprotein expression plasmid vector with a *piggyBac* transposon gene-delivery system [21,22]. The system subsequently enabled complete elimination of the exogenous reprogramming factors in mouse cells but evidently not yet in humans. More recently, Kim *et al.* have generated iPS cells by directly delivering only the necessary reprogramming proteins (Oct4, Sox2, Klf4 and c-Myc, each fused with a cell penetrating protein), thus avoiding the existing requirement for genetic material [23]. These types of development bring closer the time when clinical application of iPS cells may be possible but, compared with the accumulated knowledge of embryonic cells, much basic work remains to be done. It is also the case that with the transposon approach, and even with small molecule induction, the regulatory authorities are likely to consider this a form of gene therapy. This will have consequences in terms of the length of trials required and the long-term follow-up of patients. An encouragement comes from a study by Hanna *et al.*, which demonstrated treatment in a humanized sickle cell anemia mouse model with iPS cells generated from autologous skin, although here the method was combined with additional gene therapy [24].

One of the concerns about using autologous cells as a source of such iPS cells has been with aged patients. They are likely candidates for regenerative medicine for chronic degenerative conditions but their cells might not have a capacity to generate iPS cells. In this respect, a recent paper on iPS cells generated from a patient with amyotrophic lateral sclerosis is interesting [25]. The authors were able to generate such cells from an 82-year-old woman that met all the standard tests for iPS cell formation. As Park *et al.* note, it is becoming possible to use the method to derive pluripotent cells from patients with known diseases and to use these initially to study better molecular medicine treatments [26]. If clinically safe methods emerge, placing appropriate genes in the patient's own pluripotent cells could lead on to cell-based therapies.

For autologous cells derived by iPS cell methods, the possibility of teratoma formation is a regulatory issue in terms of proof of absence, just as it is for embryo-derived allogeneic cells. Indeed, as noted earlier, the genetic modification also raises the possibility of further complications. For autologous material it may be of value to determine ways of taking adult cells back just sufficiently far towards pluripotency that they can be expanded and switched to desired cell types. Then, because the therapy would be for a single patient, the extent of expansion would not necessarily have to be particularly large. Furthermore, the particular batch of iPS cells will only affect a single patient rather than the large number with allogeneic iPS cell or hESC therapy. Mikkelsen *et al.* have summarized some of the factors that define reprogramming of somatic cells to a pluripotent state [27]. Much earlier, Boukamp *et al.* described transdifferentiation from epidermal to mesenchymal phenotype [28]. This type of study may in time be a basis for preparing a suitable intermediate, although Graf has commented that we do not know enough yet about how cells transit from one phenotype to another [204]. Graf doubts that the cells transiently acquire an embryonic stem cell-like state but they may go back to the stage of a common precursor. This could be very helpful in avoiding the risk of teratoma formation, although not necessarily of ectopic tissue. Zhou *et al.* have reprogrammed adult pancreatic exocrine cells in mice into β-cells *in vivo* by using three transcription factors expressed in adenoviral vectors [29]. They noted that the speed of reprogramming was faster (from 3 days) than the original fibroblast reprogramming to iPS cells (7–30 days) and ascribed this to the fact that pancreatic exocrine and β-cells are closely related cell types and share much of their epigenomes. As with conventional gene therapy, it is currently unclear whether safe clinical procedures will be possible but the principle of converting closely related cells is further strengthened by this demonstration.

Potential disadvantages of allogeneic cells

In terms of disadvantages, allogeneic cells pose the problem that they will be foreign to the immune system of the recipient. Areas such as the brain had been thought to be immune privileged, however, there is evidence that immune responses can occur, albeit in a modified form [30]. There are also some indications of progress. For example, Robertson *et al.* have shown that although embryonic stem cell-derived tissues in mice are immunogenic, their inherent immune privilege promotes the induction of tolerance [31]. To achieve the latter they used nondepleting

monoclonal antibodies specific for the T-cell coreceptors, CD4 and CD8. Nevertheless, the same team with other coauthors [32] reviewed the broader recent developments in protecting pluripotent cells from the effects of alloreactivity, in other words, the immune response to allogeneic cells and the conclusions are not particularly hopeful; indeed, Fairchild *et al.* concluded that, "…constraints of alloreactivity will doubtless prove the most unyielding" [32]. One alternative is to derive a minimum number of lines to provide a reasonable match for most patients [33,34]. For example, it is estimated that 150 randomly selected donors or ten 'super donors' (donors homozygous for common HLA types) could provide a beneficial match for 38% of patients [205]. However, this will still require use of some immunosuppression. A variety of other routes are being taken to deal with this issue. In one bold approach, Tissera (Tel Aviv, Israel), deploying technology from Weizmann Institute of Science (Rehovot, Israel) [206], has used pig kidney tissue ('organoids') some 4 weeks of gestation beyond the embryo stage, which was implanted into mice with minimal immune response, because the highly immunogenic component of the blood vessels (endothelial cells) were only derived through *in vivo* vascularization by the recipient animal. Highly disparate xenographic transplants have been suggested as potential therapeutic solutions for both kidney and pancreas replacement using similar embryonic donor organs (primordia) [35–37]. Novocell Inc (San Diego, Ca, USA) is taking the route of cell encapsulation, which is a pragmatic option to avoiding an immune response. In such a situation, where the implanted cells do not have to undergo functional engraftment and are relatively easily engrafted in the abdomen, this could yield a valuable outcome even given a need to replace the cells at an interval of a few years. The field has recently been reviewed [38]. It is notable that Lindvall and Kokaia assume part of their success in treatment of Parkinson's disease via transplanting of human embryonic mesencephalic tissue from aborted fetal material to "strictly controlled immunosuppressive regimes for 1–2 years after transplantation" [39]. This compared with 6 months or lack of immunosuppressant in other trials. Extrapolation from such material to embryo-derived cells would be a considerable step but it may suggest a need.

There are indications that extended culture of embryonic cells to be used as a source of allogeneic material is associated with an increasing number of abnormalities. For example, Liang *et al.* have shown that mouse ESCs accumulate gains and losses of millions of base pairs in routine culture [40]. The copy number variation was shown to arise anew after only a limited number of mitotic divisions in culture. Although the effects of such changes were not explored they could have an impact on use for therapy. Lefort *et al.* and Spitz *et al.* have observed similar abnormalities in man [41,42]. Recently, Werbowetski-Ogilvie *et al.* characterized variants of human cells with high proliferate capacity, which revealed genetic aberrations [43]. Although the cells were not malignant, the results indicate the need for careful functional characterization. In addition, for hESCs, Draper *et al.* observed karyotypic changes involving the gain of chromosome 17q in three independent hESC lines on five independent occasions [44]. They noted that transplantation therapies in which aneuploid cells occur, in other words, with an abnormal number of chromosomes, would be detrimental. On the other hand, Rosler *et al.* found hESCs to be karyotypically normal for well over a year in culture if carefully maintained [45]. Pera commenting on Draper *et al.* related it to the broad picture and noted some caution and the need for further work [44,46].

The possibility of actual teratoma formation with embryo-derived cells is critical (**Box 1**) [47]. The issue of possible 'cancer-producing tumors' has been a contentious one with respect to embryo-derived cells [6,48–50]. Recently, Blum *et al.* have shown that hESCs transplanted from eight lines into immunodeficient mice formed teratomas (benign), whereas mouse stem cells from four lines formed teratocarcinomas (cancer) [51]. These differences seem to have contributed to the debate and the expectation is that the potential risk in humans is one of benign tumor formation. However, formation in a confined and inaccessible space, such as the brain, would be serious. It is likely that regulatory sensitivity, especially given the political issues surrounding embryo use, will make it necessary to define agreed procedures for establishing absence of teratoma-forming potential. There is a recent report of a child, who received a rather ill-defined 'stem cell preparation' as an injected material in the brain, developing benign tumors [52]. However, the condition treated, ataxia-telangiectasia, may predispose to tumor formation and without access to precise details of the original procedure, which was unavailable to the authors, this report does not seem to be a general pointer other than to the need for patient safeguards such as strict regulation and transparency.

Evidence recorded in transcripts of an Advisory Committee meeting on safety concerns for hESC-derived material in human trials of companies by the US FDA is of particular interest [53,54,207]. Lebkowski of the Geron Corporation (Menlo Park, CA, USA) in particular showed detailed rat data on the spiking of their embryo-derived human cells for spinal cord injury with defined numbers of undifferentiated human embryonic cells. This study indicated that at approximately 5% and above of added undifferentiated cells there was evidence of teratoma formation but not below this level. The site of injection was also a factor, as was the presence of aggregates of these cells. Lebkowski also cautioned that studies of human cells in animal models are not easy to interpret. In answer to questions Lebkowski indicated that cell sorting was not done and instead the production of the correct cell rested on appropriate differentiation. She also noted that their experimental results indicated that below approximately 5% there was a cutoff for teratoma formation; however, they had evidence that it is probably the absolute number of undifferentiated cells that matters rather than the percentage. This too will, of course, vary between small animals and humans. It is also the case that the short lifespan of animals such as mice and rats limits the length of study possible. A further potential safety concern discussed in the FDA meeting was that residual undifferentiated cells may migrate from the site of administration such that inappropriate differentiation may lead to ectopic tissue that could have tumor-like effects or disrupt function. The overall impression of this and the other two detailed company presentations is that this is difficult science. The initial clinical hold on an Investigational New Drug (IND) application for Geron's proposal in relation to spinal cord injury is indicative of the potential delays that pioneers face, particularly with embryo-derived cells. The delay was temporarily ended in January 2009 [55]; however, owing to an increased frequency of cysts at the injury site being observed in their ongoing animal studies, the FDA put the clinical trial back on hold [208]. In terms of risk, it is worth recalling that Kondziolka *et al.* conducted a randomized Phase II trial using neurons derived from a teratocarcinoma (embryonal carcinoma) cell line with stroke patients, which is a particularly severe test [56]. The clinical trials were conducted after animal studies with the human cell line in rats, mice and monkeys of up to 14 months duration, which demonstrated no clinically important toxicity and no tumor formation. In animal studies, neuronal cells integrated with the host brain sent out axonal processes, released neurotransmitters and demonstrated typical neuronal proteins. The animal model of stroke caused reproducible learning and motor deficits, and the injection of neuronal cells resulted in a return of learning behavior retention time and motor function. The human Phase II results demonstrated safety, feasibility and suggested possible gains but were not statistically significant enough to do more. Although the subject of some commentary [57,58], the trial is a pragmatic counter to what at some points was a rather academic discussion in the FDA meeting and it will be important not to lose sight of the need to balance risk and benefit for patients with a very poor quality of life and with no effective alternative in prospect. To a degree this is reflected in a subsequent paper from an FDA source [59], although prior to the Geron decision this left open the issue of where the regulatory body would choose to strike the balance of risk and benefit in trials. Regulatory dilemmas are not restricted to embryo-derived stem cells and von Tigerstrom has summarized some of the major issues [60]. For example, for autologous therapies, the concept of lot release testing may not be feasible in the classical sense. More broadly, von Tigerstrom noted the great disparity between individual national regulations for cell therapies.

In terms of removing potential teratoma-forming cells from products, sorting of potentially harmful cells is being applied not only to stem cells [61], but also [62] to removal of such cells in allogeneic stem cell transplantation. There are now software systems allied with advanced fluorescence-activated cell sorting (FACS) methods that have the capacity to detect and potentially destroy very rare cells and these are being explored to remove tumor cells from the bloodstream or bone marrow [63]. In principle, they might be applicable to validatable removal of potential teratoma-forming cells. In the biopharmaceutical sector, validation of the removal of adventious viruses from, for example, media components is achieved by spiking in a known amount of virus and showing that the number of log cycles of removal through several separation procedures means there will statistically be none in the finally purified product (see Titchener-Hooker *et al.* for references to scale-down methods used [64]). Were it deemed to be necessary, a similar approach might be applied for human cells. However, as Mollet *et al.* have demonstrated, current FACS designs cause cell damage by shear effects in the coned constriction leading

to the cell-scanning tube [65]. The damaged cells can be distinguished and so could potentially be removed but this loss of yield at the last stage is not desirable. With several of the current generation of commercial FACS machines being capable of modification to allow GMP operation [66,67], hopefully the necessary, and apparently feasible, manufacturer's redesign will occur. The sorting speed of current FACS machines, which are geared principally to analysis, would also be limiting if the quantities needed for therapy are large [68]. It is quite possible that if the principle of FACS for purification can be established it would be feasible to design purpose-built process sorting using this principle. This could, for example, be multichannel. The problem for such systems is always that there needs to be a market, or strong potential, to justify the high cost of development for the equipment manufacturer. This suggests that advances may occur with therapies needing very small quantities of cells where existing analytical FACS could suffice. For clinical material, the prime problem of FACS-based methods is the necessity of labeling the cells. Some such as retinal pigment epithelial cells that are targeted at the prevention of age-related macular degeneration do have characteristic pigment spectra but such cases are relatively uncommon [69]. Labeling would be acceptable if rare contaminant cells could be selectively marked and removed, because such attachment of labels would not matter to the product if it was highly specific and without label leaching. A similar logic applies to techniques using antibodies bound to magnetic particles and depletion of T cells for allogeneic stem cell transplantation provides an exemplar [62]. The monoclonal antibody would need to be of clinical grade (i.e., GMP manufactured) given concerns about murine adventitious virus transfer to human cells. There is also the issue of residual antibodies causing immunological responses in patients. As an alternative to such approaches, it has been suggested [70] that sphingolipids can eliminate teratoma cells from neural cell grafts. However, Brimble *et al.* observed no effect of glycosphingolipids SSEA-3 and SSEA-4 on teratoma-forming capability [71]. Efforts to destroy remaining embryonic cells with tumor-forming potential by inserting an appropriate suicide gene (e.g., Jung *et al.* [72]) illustrate the growing closeness of genetic and human cell technologies. This technique, reprogramming to iPS cells and transdifferentiation using means to alter epigenetic tendencies emphasize the links. In that situation it is as crucial as ever that scientists and clinicians aided by bioprocessors are allowed freedom, albeit tightly regulated, to explore all the options and their permutations [73]. It is known that hESC sources for all allogeneic cells have marked differences in their propensity to differentiate into specific lineages [74] and deriving and screening of such lines to a level acceptable to the regulatory authorities is a major issue. The problems of teratoma formation may be eased if they can be shown to be associated with particular markers [75] because this could further encourage negative affinity methods to be applied to bind such marked cells. It must also be noted that potential adverse effects are not restricted to embryonic cells. For example, Breitbach *et al.* observed bone formation in infarcted heart tissue in mice on injection of bone marrow cells [76]. By contrast, no pathological abnormalities were observed with purified hemopoietic progenitors.

Where teratoma formation is hard to address it is possible that an interesting approach described by Timmers *et al.* may be useful [77]. They showed that mesenchymal cells derived from embryonic cells produced over 200 proteins and that this cell-conditioned medium alone gave a promising outcome in a large animal model of myocardial infarct reduction. The fact that the cells were removed prior to use means the danger of teratoma formation was avoided. Then, as noted earlier, where limiting the natural disease progression of an acute condition is the goal, a preparation of this kind may have a real benefit even if regeneration of the damaged tissue is not possible. iPS cells may pose more risk of cancer formations due to subtle differences in their epigenetics but the situation is as yet unclear [2,6]. The relevant background is described by Nishikawa *et al.* and Knoepfler [78,79].

For allogeneic cells, the issue of immune response impinges on that of possible teratoma formation. Dressel *et al.* investigated tumorigenicity of mouse embryonic cells and of *in vitro* differentiated neuronal cells and found it to be controlled by the recipient's immune response [80]. They concluded that, in their study, differentiated cells must contain a tumorigenic cell population that is not present among ESCs. Roy *et al.* examined engraftment of hESC-derived dopaminergic neurons, enriched by coculture with telomerase-immortalized midbrain astrocytes [81]. While the method gave promising results in terms of the formation of the required subtype of neurons, there proved to be a core of cells with few dopaminergic neurons that were potentially tumorigenic. This was evidently due to incompletely differentiated cells. If the recipient of embryo-derived allogeneic cells required

immunosuppression, it is possible the cell concentration at which teratoma formation became an issue could change. Immunosuppression is also a tumorigenicity risk in its own right as a potential long-term hazard [82]. To preserve balance, Condic and Rao noted that when iPS-derived autologous cells are used the frequency of tumor formation may be greater because with autologous material there will be no immune system rejection [9].

For allogeneic cells, it is possible that a line chosen could entail a disadvantageous trait. However, given the therapeutic target it is possible to choose an embryonic cell line which lacks known genes that can cause a predisposition to the condition being treated. Thus, in treating age-related macular degeneration, cells with variants in the gene for complement factor H will be avoided because they have been implicated as strongly associated with the disease [83,84]. There remains the issue that some diseases are evidently influenced by a number of as yet undefined genes. When the therapy is principally to be applied to older people, as is the case with stroke, heart failure and Parkinson's disease, the presence in the original embryonic source for allogeneic cells of such, as yet unknown, genes predisposing to later diseases may be less of a concern in risk-versus-benefit terms. However, with such cells derived from embryonic lines for treatment of conditions in young people with a long life expectancy this is a more significant issue. There are alternative approaches to achieving safer cell sources in terms of genetic predisposition. One would be to use somatic cell nuclear transfer [85], although, as Fairchild *et al.* noted, the mitochondrial genes remain the preserve of the donor [32]. If the cell nucleus is derived from a healthy individual with no known genetic risk factors for serious medical disorders, this would give the maximum opportunity to produce a therapeutically beneficial somatic cell nuclear transfer cell line. A similar logic applies to iPS cells [19] from fibroblasts. It is worth noting though that fibroblasts, which are generally regarded as rather stable in terms of genetic definition, can entail mutations that cause disease [86,87]. Age-dependent changes [9] may also apply to cells derived from adults. Advances in human genome analysis will eventually allow greater screening but disease risks arising owing to complex, multifactorial and polygenetic causes will be more slowly characterized.

For embryo-derived allogeneic cells, the issue of checking for transmissible diseases such as HIV raises difficult issues in consenting donors of the embryonic cells. For example, screening in relation to IVF as a source in the UK will not as things stand provide sufficiently detailed information on risks of virus presence. Although it is resolvable in future, there has also recently been concern over the potential inadequacy of past patients consenting in the provision of embryonic cells [88,89]. This could possibly restrict the number of existing lines that may be used commercially and establishing new lines would then be time consuming. The original draft guidelines of the US NIH for human stem cell research [209] would preclude their funding research on existing embryo-derived lines since they would not have met the proposed rigorous consenting requirements. While less restrictive than prior US government policy this would have significantly disturbed the continuity of research because such lines have a large amount of accumulated knowledge that can be built up upon. In the end, pragmatism appears to have prevailed [90]. In addition, the California Institute for Regenerative Medicine (CIRM) as well as the International Society for Stem Cell Research (ISSCR) are both involved with new guidelines to ensure a better format for future consenting [91,92].

O'Rouke *et al.* have recently described the many detailed issues involved in establishing centralized banks for hESCs [93]. As they note, it is hard to escape the conclusion that governments and perhaps very large medical charities are the only likely custodians of publically available banked embryonic cells. In countries such as the USA, where companies can establish their own banks, the issues differ but are no less demanding. One potential problem O'Rouke *et al.* briefly mentioned is that if, as in the UK, companies are essentially obliged to place lines in the national bank they are then available to others, albeit with intellectual property limitations [93]. If a very limited study on such a line is published by a group other than the commercial originator, any negative results could have a damaging impact on commercial prospects even when a full, more thorough, study published earlier is more positive.

A significant issue with respect to the derivation and culturing of hESCs from which to derive allogeneic cell products is the general requirement for the use of animal cells and products in cell culture [94]. This concern is due to the potential risks of graft rejection and zoonosis transmission [95]. It has generally only been possible to derive human embryonic cells on a feeder layer of murine embryonic fibroblasts.

There are reports of derivation using human feeder layers [96,97]. However, the continued use of murine embryonic fibroblasts by most groups for initial derivation suggests continued caution is still required. Recently, it has been reported that human iPS cells can be generated using immortalized human feeder cells and this may represent an eventual gain. However, a key issue will be the capacity of the feeder layer to promote undifferentiated growth [98]. Clinical products manufactured on murine feeder layers have been accepted by the regulatory authorities including the FDA [210], for example in the commercial product Epicel® [211] used to treat severe burns [99]. That is because human epithelial cells demand coculture. This product has been in use for 20 years with no adverse effects [100]. However, the treatment helps prevent almost certain death so is not a clear precedent. The FDA will require long-term follow-up of patients for commercial products where the embryonic cells are derived on murine feeder layers because they fall within xenograft rules [6]. Thus, it does pose an additional burden. This will be borne, for example, by the first embryo-derived stem cell therapy to receive IND application clearance. The human cells for Geron's proposed therapy for spinal cord injury were derived on mouse feeder layers.

Aside from these potential disadvantages there is a practical challenge in that a large amount of initial testing is needed. Such characterization is especially critical because of the potential to treat many patients with cells derived from a single source. With autologous cells any possible harm due to the cellular component is limited to the individual patient.

Potential disadvantages of autologous cells

While allogeneic lines pose risks there are situations where it may be desirable to avoid autologous cells. For example, if the patient is already known to have a genetic mutation or predisposition that has led to a disease it may be wise to avoid their own cells as a source or the additional challenges of inserting correcting genes if these are known will have to be addressed. Type 1 diabetes is an autoimmune disease, therefore allogeneic replacement of the β-islet cells could be less prone to the mechanism of loss that has befallen the patient's own original islet cells. Interestingly, in spite of this predisposition, identical twin to twin pancreas transplants (syngeneic) for Type 1 diabetes have been successful, with moderate immunosuppression [101]. As noted elsewhere, allogeneic cells derived from embryos may carry genetic predispositions but it will cost less to define known genetic factors with these for a cell line potentially capable of treating many patients.

If the condition to be treated is truly an acute emergency then there will not be time to expand autologous material (**Box 1**, disadvantages). Autologous cells are generally used in the treatment of severe burns because the immune response here is severe with regard to allogeneic cells [102]. Efforts have been made to use allogeneic material but as yet there is not enough evidence to encourage its use for burns patients [212] but, as noted below, an 'intermingled' combination of allogeneic and autologous cells has shown effectiveness. In the case of severe burns, patient by patient decisions are made as to how long to continue expansion before implanting autologous cells on the wound. In all autologous cell therapies, and especially critical ones of this kind, the premium on consistent process success is very high because inherently there will be a significant delay if the cells fail to meet specification.

Autologous cells harvested in a clinic distant from the cell expansion site entail an extra transportation cost and the initial biopsy will add clinical and possibly surgical theatre time not required for allogeneic material. However, on occasion, as in the biopsy of chondrocytes for treatment of a damaged knee, this is an opportunity for necessary examination to check whether, for example, structural degeneration is likely to preclude a good outcome [103]. It is also likely that some simple procedures such as collecting skin fibroblasts via biopsy can be done as outpatient or clinic procedures.

Compared with allogeneic cells the throughput with autologous cells will tend to be modest with current technology, but fortunately in some cases such as acute burns that is less of an issue. It is probably a pointer that the Organogenesis allogeneic bilayered tissue-engineered skin (Apligraf) has now exceeded 250,000 units shipped [213] whereas the autologous Genzyme chondrocyte product (Carticel®) for sports-related knee injuries has treated approximately 14,000 patients. The latter number does also relate to the need for the knee to be otherwise healthy and this figure represents a sizeable proportion of the potential market. A disadvantage of autologous cells is that each patient's material must be separately processed. At present this entails the attention of a skilled technician in manual procedures for each unit of therapy, which makes the costs high.

Therefore, although small numbers can be dealt with in public sector clinical centers where there is often a high concentration of such people, it is less easy in a commercial setting. Retaining such staff to work on large numbers of repetitious procedures is not easy. Automation, at least for simple steps, may be an option as the field matures but capital expenditure is high, and investors and equipment manufacturers will demand an assurance on markets before wishing to spend large sums. In addition, the variability of individual patient's biopsy material is evidently a major reason why the processing of autologous material such as chondrocytes has remained manual [104]. We have noted elsewhere [105] that identification and tracking of individual patient's material throughout the whole process from biopsy through expansion to implantation is a critical issue for manual and automated procedures with autologous cells. Its failure is a potential disadvantage.

There are a growing number of both autologous and allogeneic therapies based on bone marrow-derived cells and similar cells derived from, for example, adipose tissue. With all these materials the variability of the source material is probably the greatest current concern (**Box 1**, disadvantages). The issue is most pronounced for autologous therapies where the cell composition and level varies with each patient, in contrast to treatments where material from healthy donors is used for a large number of allogeneic therapies such as immunomodulation cell therapy for Crohn's disease. The difficulty is reflected in the problem of achieving a well supported result in the trials of autologous bone marrow-derived cells in heart failure [106–108]. The latter reviews defined the reasons in detail. True cardiac regeneration is demanding in requiring the implanted cardiomyocytes to establish gap junctions that support electromechanical connections with the recipient's cardiomyocytes, thus producing the necessary functional syncytium. This syncytium would facilitate graft–host synchronized heartbeats, which are critical for the restoration of normal contractility. Such integration has not yet been achieved with adult stem cells. Bianco *et al.* have recently summarized the history of what were called mesenchymal stem cells and are now usually described as mesenchymal stromal cells [109]. They provide a detailed analysis of the complexity of the material and of the dangers of drawing oversimplified conclusions. The issue of defining the nature of cells also applies to similar sources such as adipose tissue [15]. In some instances, it appears that the autologous cells may be providing paracrine factors [110]. The therapeutic capacity of added cells that only produce such factors is not necessarily trivial and there are several reasons why they may not easily be replaced by equivalent products from the biopharmaceutical sector. One is that there is a relatively complex mixture of molecules that is hard to produce except by methods equivalent to those already being used in the regenerative medicine process. A second reason is that, although the cells may not be integrated with the local tissue, they may function to produce factors over a period of time. In principle, this can be achieved by a variety of immobilization procedures but the cells may be a more natural source. Finally, it is possible that, in at least some instances, there is 'cross-talk' between the added cells and those of the recipient tissue [111]. Therefore, while such factors probably cannot stimulate cell regeneration beyond some limit it may be adequate to lift performance above a key level for patients such as those suffering heart failure and prevent the damage from escalating to end-stage heart failure. The severe demands in creating fully functional cardiomyocytes are not only a challenge for bone marrow-derived cells and the like but equally for hESC-derived material [112]. Laflamme *et al.* achieved encouraging results but studies of human cells in small animals are a particular problem here, as Rubart and Field have pointed out, because of the higher beating rate of the heart, which will likely have an adverse effect on human cells [113,114].

The complexity in all stem cell systems, which is acutely reflected in bone marrow-derived material, is beginning to yield to analysis. Muller *et al.* provide specific evidence for marked differences between embryonic and other pluripotent cells and a variety of human stem cell lines including adult ones [115]. By sophisticated analysis of gene-expression profiles they were able to classify the cells and also used bioinformatics methods to uncover a protein–protein interaction network distinctive to pluripotent cells. While the authors note this is 'work in progress' it does begin to suggest how stem cells can be more systematically classified and related and could begin to address some of the existing empiricism.

In certain instances there may be a justification for mixing autologous and allogeneic cells. The method of 'intermingled skin grafting' of allogeneic material with small islands in punched holes of autologous material was pioneered in China in the 1960s. Recent literature [116,117] suggests that it has value in disaster situations but, from a cosmetic point of view, transplantation

of autologous keratinocytes results in a better aesthetic homogeneous texture. Falanga has recently described a treatment for scleroderma, an autoimmune disease, in which a patient's bone marrow cells was applied to their ulcers using fibrin [214]. The wound was then covered by an allogeneic bilayered substitute derived from neonatal foreskin.

Broader issues

The ethical, legal and religious issue of embryonic cell use in a number of countries remains a constraint on use of this class of allogeneic cells. This may mean that, until iPS cells or a similar development such as controlled transdifferentiation occurs, the focus on natural autologous cells will remain strong. It is against this background that such countries or regions may proceed more via hospital-centered personalized patient services. Because the role of the clinician is so crucial in regenerative medicine [73] their moral and ethical beliefs are also an important factor such that even when these constraints do not impact on production they can affect cell implantation. Here, it may be necessary with embryo-derived material to have the same 'conscientious objector' status as is allowed in the UK with obstetricians who oppose termination of pregnancy [215]. Cultural considerations in Japan, where use of donated cadaveric tissue although now legal is not widely favored, was a driver for the derivation by Yamanaka and colleagues of iPS cells from human fibroblasts [19] so that at least cultural considerations have led to a broadening of the options potentially available.

It is likely that one of the most important nontechnical issues will be the effect of changes in policy by the US Federal Government. The stimulus to publicly funded research on embryo-derived allogeneic cell-based regenerative medicine may in time be considerable in the country with the largest potential initial and long-term market. This is also likely to influence the position of the pharmaceutical industry, which until recently had been generally cautious of using human embryo-derived cells even for safety testing of potential new drug compounds. That situation has now changed, although resistance by some in the population will remain a significant factor in the USA, as in some EU countries where major companies must consider the impact on their share price and sales of molecular medicines. Nevertheless, it will lend impetus to the action of companies such as AstraZeneca, GlaxoSmithKline and Johnson & Johnson, Novo Nordisk, Roche and Pfizer in either use of pluripotent cells in drug discovery and toxicology or regenerative medicine studies. A reduction in NIH restrictions on funding coupled to increased US government research spending will encourage more major companies to enter the field or purchase startups and this could accelerate the advance of allogeneic cell technology. Such entry of pharmaceutical or medical device companies will profoundly change the nature of regenerative medicine.

In the 1980s, with rare exceptions such as Eli Lilly, Roche and Novo Nordisk, pharmaceutical companies were initially sceptical of the potential of therapeutic proteins that had to be injected and were relatively more difficult to produce [118]. Now with a $100 billion market these reservations have proven to be incorrect. Human cells represent a larger step both in technical terms and commercially because they are potentially a single-use definitive treatment. However, given the importance of the conditions that could be addressed and for which molecular medicines are not very effective it is likely that the recent entry of several major pharmaceutical companies, such as those cited earlier, will encourage others. Given that the combination of scientific and regulatory obstacles is considerable, it will be hard for hospital-centered or biotech business models to progress through complete clinical trials for human cells with any but basic therapies. In this situation, the position of large pharmaceutical companies will be crucial because, at least in the past, they have had the resources and patience to reach back deep into science and then carry through the extended and high-risk task of development. It could be that clinical successes with autologous adult cells, even if commercially less attractive, might help by providing added conviction that regenerative medicine is the basis of an effective therapy for previously poorly addressed conditions. Because of the potentially very wide use of human cells, attention to defining their nature is critical. The biopharmaceutical protein industry has come to learn that 'the process is the product' and, for example, changes to the formulation and different routes of administration of erythropoietin raised the risk of antierythropoietin antibody formation and increased the incidence of pure red cell aplasia [119]. When the products are living cells, which are very expensive to prepare, processing knowledge will be as critical as scientific insight. Indeed, without a reliable means of producing cells consistently even the earlier stages of the development pathway will be unworkable.

We have commented on the problems associated with the complexity of bone marrow material and related sources. However, it has to be acknowledged that the effective functioning of particular types of cells is often strongly influenced by the presence of other types. In general, the pharmaceutical industry prefers pure substances because even then the assurance of quality is demanding. Nevertheless, it may be necessary both for stability during processing and to achieve the desired function to consider cell mixtures. Then, the aim will be to minimize the cell types present and maximize the characterization.

An issue that will have a powerful influence is the existence of key patents. This bore on the earlier development of therapeutic antibody technology and it will inevitably affect the choice of approach in regenerative medicine. For example, the key Wisconsin Alumni Research Foundation (WARF) patents for derivation of human embryonic cells have been narrowed but stand [216]. However, they have been disallowed in the EU on the basis they involved destruction of a human embryo [217]. These patents expire in 2015 [120] but the annual commercial licenses required in the interim are an obstacle, particularly for startups. If it were possible to safely re-engineer adult cells back to progenitor rather than pluripotent cells this could represent an alternative that did not require this WARF intellectual property. On the other hand, were it to be ruled that the original WARF patents apply to iPS cells this would equally mean that after 2015 the field might be clear of master patents of this particular type. Yamanaka and others have filed patents on iPS cells, the publications of which are now beginning to appear from the various national patent offices [218].

In assessing the cost comparisons of autologous versus allogeneic cell therapies it is important to have in mind the costs of the cells versus associated clinical services. It is certainly the case that autologous cells will have an additional biopsy cost and, in an example such as harvesting nasal ensheathing cells to treat spinal cord injury [121], this is complex and costly, even if absorbed as a hospital cost. In other instances the main clinical implantation procedure and delivery device costs will often represent a major element so that neither autologous nor allogeneic cell bioprocessing may necessarily be the decisive costs for the payer. Even when producing the cells is a key part of cost the processing is of course just one element alongside characterization, quality control, quality assurance and all the issues concerning safety. Based on the experience of developing and producing a blood vessel, McAllister *et al.* of Cytograft Tissue Engineering (Novato, CA, USA), have commented on cost issues with much insight into the very demanding requirements to bring through such human materials to commercialization [122]. They make it plain that with even tighter regulation and greater pressures on cost–effectiveness by reimbursing agencies, the pioneers of regenerative medicine will need to be very disciplined in their approach. Although in theory allogeneic therapies might be capable of greater returns, particularly if they treat conditions for which there is currently a large unmet clinical need, the risks in terms of complications such as immunorejection and teratoma formation make this no easy alternative.

Over all the issues of advantages and disadvantages of autologous and allogeneic cell therapies lies a broader one. This is the question of when the knowledge of either cell approach to therapy is adequate to proceed to clinical trials with a reasonable prospect of success. There are those who make the case that as yet the knowledge is too incomplete (see Bakay [58]). It is certainly true that information on the fate of implanted cells is limited. However, as has been well argued in the case of stroke clinical trials [56], it will only be by initially limited clinical trials that some key scientific and clinical issues are defined. One helpful pointer in this regard is the long-term observation of Parkinson's disease patients treated with fetal-derived material. Even though after a period of years some investigators have observed Lewy bodies characteristic of the disease in autopsied graft material of treated patients [123], the majority of the graft was unaffected. Other investigations found no altered pathology even after 14 years postimplantation [124].

Future perspective: the potentially decisive issues

From the above discussion it is possible to recognize a number of issues that could be decisive in influencing the balance of advantage between allogeneic and autologous cells (summarized in **Box 2**). Some are scientific and others are much broader. The issue of immune response to implanted cells has been placed highest because it could have a profound impact on the use of allogeneic cells requiring all therapies to use immunosuppression and/or the requirement for successful immunotolerance strategies. As noted earlier in relation to the work of Lindvall and colleagues [39], nonrejection has been achieved

Box 2. The potentially decisive issues.

- Solutions to the immunological challenges.
- Cell purity issues such as being able to prove absence of contaminating cells that might form teratomas and teratocarcinomas *in vivo*.
- Resolution of the scientific uncertainties surrounding bone marrow stromal cells and similar materials.
- The challenge for induced pluripotent stem (iPS) or similar cells to meet clinical safety requirements.
- The practical outcome of any changes in government/state policies on embryonic cells.
- Key patents, changes to patent law and any international harmonization.
- Multiple entries of major pharmaceutical or medical device companies into commercial regenerative medicine in order to more fully exploit the technology.
- The relative cost of universal (allogeneic) cell products and their associated characterization versus the clinical service model deploying patient-specific (autologous) cells.

over more than a decade with allogeneic material in the brain. Here, and in other less long-term studies, there is an indication that immunosuppression for a period of several years may possibly suffice. The issue is one of great importance.

The issue of possible teratoma formation is as large for pluripotent cell-derived material, although not for other allogeneic therapies, as that of immune response. However, it should be more susceptible to technological solutions, as described earlier.

The uncertainties over the precise nature of bone marrow stromal cells and related materials will steadily yield to scientific study. In terms of commercial developments, a glance at business news might suggest that there are just two kinds of regenerative medicines, bone marrow derived and other. As noted earlier, there does seem to be a genuine role for such cell complexes. However, the inference of 'cure all' that hovers over this material and related cell complexes could be damaging if claims cannot be supported in rigorous trials [125].

The rapid advances in the science of iPS cells represent a potentially very disruptive force in a technology sense for both allogeneic and autologous cells. However, as McAllister has emphasized [Pers. Comm.], there are dangers in those involved in translation – jumping from established science to exciting but as yet uncertain scientific territory. The effort of translation and its risks are so large that there does need to be a solid foundation from which to proceed. That said, unless there are unforeseen problems, iPS-derived cells will be a key future factor.

The attitude of governments in some EU countries and especially in the USA will have a great effect on embryo-derived allogeneic cell therapies, which inherently are likely to be ready for routine clinical use well before iPS cell-derived material. The US situation now appears hopeful but, as in continental Europe, there remains major opposition. Embryo-derived cells will remain a gold standard for the foreseeable future at least in terms of scientific understanding. The decade of investment already made in research and preclinical effort also should not be wasted. For at least those of the public with an open mind, the hope must be that scientists and clinicians can argue for pragmatism and patience, perhaps with an acknowledgment of the desirability of avoiding embryo donation when the future situation allows. Related to this and more dominated by the attitude of some EU governments, the patentability of embryo-derived cells will influence the uptake of embryo-derived allogeneic therapy in Europe as the second most significant potential early market. Once valuable therapeutic effects are established it is hard to believe all the member states of the EU will not take up such materials.

Because of the greater ease of using autologous cells for clinician-centered activity in hospitals and clinics, plus given ethical obstacles for embryo-derived material, autologous cell trials are much greater in number than those for allogeneic ones. The entry of pharmaceutical companies is likely to change the balance in time. Their capacity to conduct large and costly trials and the closer nature of allogeneic cell therapies to molecular medicines will emphasize this tendency.

Given the astonishing advances in the last several years, one crucial conclusion to draw is that at present it is unclear which methods will come to be most significant. Indeed, the choice between autologous and allogeneic may remain dependent on application. With the need for clinicians to adapt to regenerative medicine, it is perhaps less critical which type of cell they use at present than that they accumulate experience and insight. Then, the second-generation cell therapies will be met by a medical community that knows what it is looking for and has gained the expertise to apply regenerative medicine efficiently.

There is steadily accumulating evidence of the value of human cell therapies. The indications that they can both regenerate in chronic conditions and limit disease in acute ones is exciting in

widening that promise. The challenge now is to work at the decisive technical issues which both allogeneic and autologous cell therapies still face and to use progress on either to demonstrate to politicians and the public that the whole field, whichever type of cell is chosen in particular cases, is worth supporting.

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Executive summary

- The advantages and disadvantages of patient-specific (autologous) and universal (allogeneic) regenerative medicine therapies
- Scope: cell- and tissue-engineered therapies
- Permanent cell-replacement therapy (chronic) and disease-modifying therapy (acute)

Potential advantages of allogeneic cells

- Universal approach similar to pharmaceuticals
- Potential for a 'block buster'
- Easier to scale-up
- Potential economies of scale
- Commercial product

Potential advantages of autologous cells

- Patient specific – no rejection issues
- Potentially quicker to clinic
- Potential for point-of-care bioprocessing
- Preferable for bioaesthetic applications
- Service model

Potential disadvantages of allogeneic cells

- Immunological incompatability
- Risk of cell abnormalities (e.g., karyotypic and epigenetic changes)
- Risk of tumor formation (e.g., teratoma [benign lesion])
- Development investment high

Potential disadvantages of autologous cells

- Variability of source material
- Challenge to expand patient cells
- High cost per patient (product of one)
- Any process failure – major therapy delay

Broader issues

- Resolution of cultural, ethical and legal issues
- Master patents, patent thicket and the need for freedom to operate (potential for patent pooling)
- Hospital-centered patient service model – embedded in existing healthcare facility
- Funding and reimbursement
- Requirement for the involvement of big pharma and the larger biotechs (analogous to biotech in the 1980s)
- Quality control (QC) and quality assurance (QA) issues

Future perspective: the potentially decisive issues

- Failure to find acceptable solutions to the immunological isssues
- Cell purity issues such as being able to prove absence of contaminating cells
- Resolution of the scientific uncertainties surrounding adult stem cells
- Challenge for induced pluripotent stem (or similar cells) to meet clinical safety requirements
- The practical outcome of any changes in public support and/or government/state policies on stem cells
- International requirement for patents and regulatory harmonization
- Significant endeavors by the multinational companies to more fully exploit the technology
- The relative cost of universal (allogeneic) cell products and their associated characterization versus the clinical service model deploying patient specific (autologous) cells

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