Current prospects for the generation of patient-specific pluripotent cells from adult tissues

'The great promise of stem cell-based therapy is to tackle the problems of modern transplantation by allowing the creation of genetically identical, healthy tissues to replace those damaged by disease or age.'

Major advances over the past 60 years have made bone marrow and tissue transplantation standard clinical therapies for a wide range of diseases. However, modern transplantation is plagued by immunological incompatibility and a dearth of suitable donor tissues. The great promise of stem cell-based therapy is to tackle both of these problems by allowing the creation of genetically identical, healthy tissues to replace those damaged by disease or age.

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Interest in embryonic stem (ES) cell-derived cellular therapies has developed rapidly in the past decade, fueled by several key scientific observations. In 1997, Wilmut and colleagues expanded on 50 years of nuclear transfer research to show that a fully developed mammal could be cloned from an adult cell [1]. This observation has since been repeated in several other species [2] and has established the general principle that highly specialized cells can be 'reprogrammed' to a state of pluripotency – the capacity to form all tissues in the body. It has also been shown that these reprogrammed cells can be propagated as ES cell lines [3–5], making them amenable to *in vitro* manipulations, including the correction of genetic defects. Advances have simultaneously been made in the directed differentiation of ES cells to create specific tissue progenitors [6,7]. In combination, these studies set the stage for proof-of-principle demonstrations of ES cell-based therapy in murine disease models. In a 2002 report, Rideout *et al.* reprogrammed skin cells from an adult immunodeficient mouse, derived an ES cell line, and corrected the underlying genetic defect in the cells [8]. The 'repaired' ES cells were differentiated *in vitro* into hematopoietic progenitors and infused back into the mouse to reconstitute its immune system.

In a similar fashion, it is envisioned that patient-specific ES cell lines will be derived and serve as a source of genetically matched replacement tissues for a myriad of neurologic, endocrine, hematologic and other disorders.

While the manipulations described above are feasible in mice, significant technological and ethical hurdles hinder translation of this approach to human clinical therapy. One of the main challenges is at the very first step, the process of generating pluripotent cells from adult tissues. As a result, recent efforts have focused on improving reprogramming methods or innovating other means to create genetically identical pluripotent cells. This editorial describes the methods that have been put forth to date, and critically evaluates their scientific validity and prospects for clinical translation.

Nuclear transfer

Early reprogramming studies were carried out using the technique of nuclear transfer (NT) in amphibians, in part due to the relative ease of manipulating their extremely large eggs. In NT, the genetic material of a recipient egg is physically removed, and a donor nucleus from any other tissue or cell type is injected into the enucleated egg (Figure 1). In some cases, the egg is able to adopt the donor nucleus and progress through development. In 1952, Briggs and King showed that if the genetic material from a frog egg was replaced with the more differentiated nucleus of a blastula cell, the reconstructed embryo could give rise to a hatched tadpole [9]. Gurdon extended these observations in 1966 to show that when even more differentiated frog intestinal cells were used as NT donors, fully fertile adult animals were able to develop from the reconstructed embryos, albeit at a low efficiency of approximately 1% [10].

Over the next two decades, advances in micro-manipulation technology (the equipment used to remove and replace nuclei in single cells) allowed NT experimentation in mammals. This was a significant technical achievement given that mammalian eggs are 1/1000th the volume of amphibian eggs. Work by several groups in the 1980s showed that fully developed animals could be generated when mammalian embryonic nuclei

were transferred to zygotes or oocytes [10]. Further experimental advances led to the remarkable demonstration that live adult animals, such as Dolly the sheep, could be generated using adult cells as nuclear donors. This feat was repeated in several species using a variety of differentiated donor cell types, including lymphocytes [11], olfactory neurons [12,13] and even cancer cells [14].

Taken together, these experiments bolstered a fundamental principle in developmental biology: that the specialization of cells and tissues involves the selective expression of an identical genome, rather than changes in the content of the genome (with the notable exception of lymphocytes). As importantly, the studies proved that under experimental conditions it is possible to return a differentiated genome to its original zygotic state, one capable of generating any tissue in the body. Such 'alchemy' has been the main driving force behind recent interest in pluripotent cell-based clinical therapies, wherein a patient's own cells will be reprogrammed and regenerated as healthy replacements for their diseased tissues.

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What are the limitations to NT-based reprogramming for the creation of patient-specific cells? First and foremost, the technique has not been shown to work in humans. Exciting reports of NT using human donor cells and oocytes by the group of Hwang [15,16] were unfortunately found to be fraudulent [17]. Initial failures of NT in monkeys led to speculation that peculiarities in the biology of primate cells might make them impossible to reprogram [18]. Moreover, the Hwang group's inability to produce a true cloned ES line after NT of over 2000 human oocytes dampened enthusiasm that this technology might be applied to humans. However, the establishment of monkey ES lines by NT of adult skin cells was recently reported at the annual meeting of the International Society for Stem Cell Research [19]. This finding, if reproducible, should lay to rest theoretical arguments against the generation of primate pluripotent cell lines by NT, and will reinvigorize efforts to do the same in humans.

A second critical barrier to NT-based reprogramming of patient cells is the need for oocytes as the recipient cells for NT. Based on the poor efficiency of NT in most species tested so far [20], significant numbers of human oocytes would be needed to generate a single patient-specific ES line. This raises numerous issues surrounding the means of human oocyte procurement for research and clinical therapy. Improving the efficiency of NT might address these concerns: if sufficient oocytes for the production of an NT ES line could be obtained from one or a few rounds of superovulation, a family member (or the patient herself) may be able to serve as the sole donor for any given patient. However, rational improvements in the efficiency of NT may be far off, since the mechanisms by which the oocyte reprograms the donor genome remain largely unknown despite decades of research. As a possible alternative to using oocytes, a recent report by Egli *et al.* demonstrates that mitotically arrested mouse zygotes can serve as NT recipients for reprogramming [21]. On this basis, the authors propose that human zygotes destined to be discarded as medical waste in IVF clinics might be used as the target for NT instead of human oocytes. If this technique is shown to work in previously frozen human zygotes with reasonable efficiency, it may address some of the practical challenges surrounding procurement of recipient cells for human NT.

A third potential barrier to the clinical application of NT stems from the fact that generating patient-specific pluripotent cell lines by this method involves human embryos. It is highly improbable that an NT blastocyst will go on to develop into a live, adult animal [20]. Still, some feel that it is ethically unacceptable to manipulate or destroy human embryos in the course of research, even for potentially life-saving therapies. This has led in some cases to bans in government funding or on research altogether using new human ES lines created from developing embryos, whether from NT or other sources. A detailed ethical consideration of NT and other methods for generating patient-specific pluripotent lines is beyond the scope of this editorial, but is the subject of other recent contributions [22–27].

Cell fusion

Scientific advances using NT have been the major impetus for interest in pluripotent cell-based therapy in recent years. However, basic research in developmental biology has also yielded other reprogramming methods, some of

Figure 1. Proposed methods for generating patient-specific pluripotent stem cells from adult tissues.

Pluripotent cell lines may be able to be derived from differentiated somatic cells by three methods involving reprogramming: nuclear transfer, cell fusion and transduction with defined factors. Alternatively, pluripotent cell lines may be obtainable by selection and culture of male germline stem cells, or following parthenogenetic activation of female oocytes. ES: Embryonic stem cell; NT: Nuclear transfer; SSC: Spermatogonial stem cell.

which are under consideration for clinical use. In another early demonstration of mammalian reprogramming, Miller and Ruddle showed in 1976 that undifferentiated embryonal carcinoma cells could confer features of pluripotency on differentiated thymocytes when the two cell types were fused together [28]. This observation has since been repeated using pluripotent mouse and human ES cells as fusion partners for a variety of differentiated cell types [29–31]. In the fusion procedure, hybrid cells are created by electrical, chemical or biological means (Figure 1). Fused cells are selected using double drug-resistance or fluorescence marker screening, with one marker contributed by each of the fusion partners. The reprogramming event is indicated by the outgrowth of clones from the selected fusion population under ES cell culture conditions. Hybrid clones isolated in this manner have been shown to be pluripotent by their ability to give rise to tissues of all three germ layers (endoderm, mesoderm and ectoderm) as teratomas when injected into recipient mice. Several molecular hallmarks of pluripotency are also detectable in the fused cells, including the activation of the pluripotency genes, such as *Oct-4*, and activation of the second X chromosome, which is normally silenced in somatic cells.

Thus, the pluripotent cell, like the oocyte and zygote, contains the ability to reprogram a differentiated cell. However, the reprogramming event is again very inefficient, as indicated by the rarity of hybrid clone outgrowth. The mechanisms by which pluripotent cells reprogram the differentiated genome are unknown, but must involve dominant *trans*-acting factors residing in the nucleus and/or cytoplasm [32,33]. One pluripotency-associated transcription factor, Nanog, has been suggested to increase the efficiency of reprogramming after cell fusion. Silva *et al.* reported that ES cells overexpressing Nanog at approximately five times basal levels were able to increase reprogramming of neural stem (NS) cell fusion partners by up to 200-times [34]. The mechanism by which Nanog does this is unknown. Interestingly, Nanog was neither able to directly reprogram NS cells, nor increase reprogramming as efficiently if it was overexpressed in NS cells instead of ES cells prior to fusion. It is possible therefore that in this assay Nanog supported the outgrowth of reprogrammed cells, instead of playing a direct role in reprogramming. Still, it is the only factor that has been shown to enhance pluripotent cell derivation in

a fusion assay, making it an important candidate mediator of reprogramming and worthy of further investigation.

The clinical utility of pluripotent cells generated by cell fusion is limited most obviously by the fact that the hybrid cells would have double the chromosome content of normal cells. Carrying genetic material from both the pluripotent and differentiated cells would render the hybrids genetically unstable and immunologically incompatible with the donor, and thus unsuitable as progenitors for therapeutic tissues. Some efforts have been made to remove the pluripotent donor chromosomes from the fusion product after reprogramming has occurred. Matsumura *et al.* have shown that by making genetic modifications to the pluripotent donor chromosomes, they could be targeted for deletion from the fused cells [35]. It remains to be seen whether removal of all of the donor chromosomes will be possible by this method. Improvements in both the inefficiency of fusion-based reprogramming and the resulting aneuploidy will be needed if this methodology is to be translated to clinical use.

Directed differentiation with defined factors

The cell fusion experiments described above showed that ES cells contain dominant factors capable of reprogramming a differentiated genome. This observation formed the basis for attempts to identify which factors in the pluripotent cell were responsible for the reprogramming activity. In a groundbreaking 2006 report, Takahashi and Yamanaka defined four factors present in ES cells that could confer the property of pluripotency when ectopically expressed in differentiated cells [36]. The Yamanaka group began by selecting 24 ES-expressed genes that they hypothesized might have the ability to confer pluripotency based on their roles in the maintenance of pluripotency. A total of 24 strains of retrovirus, each expressing one of these genes, were produced and used for infection of skin progenitor cells called murine embryonic fibroblasts (MEFs). The MEFs contained a neomycin drug resistance gene under the control of the *Fbx15* promoter (*Fbx15-neo*), which is only active in pluripotent cells. The authors expected that if any combination of the 24 factors was able to reprogram the cells, the *Fbx15* promoter would be activated and the event would be detected by outgrowth under neomycin selection. Remarkably, after co-infection of MEFs

with all 24 strains of retrovirus, followed by neomycin selection for 2–3 weeks, rare colonies emerged that displayed morphological similarities to ES cells. By repeating the assay with different combinations of the 24 retroviruses, they found that only four factors, Oct-4, Sox-2, c-Myc and Klf-4, were required to generate these ES-like colonies (Figure 1). Lines of cells from these colonies displayed gene-expression profiles and DNA methylation patterns similar (though not identical) to ES cells. On this basis they were dubbed induced Pluripotent Stem (iPS) cells. Some iPS cell lines were able to form tissues from all three germ layers when tested both by teratoma assays and by *in vitro* differentiation into embryoid bodies. The authors concluded that pluripotency could be conferred on differentiated cells by ectopic expression of only a few defined factors.

The claim that the vast complexity of nuclear reprogramming could be reduced to four factors was naturally met with skepticism. However, less than a year later, these results have been not only verified, but also improved substantially. Three recent reports show that if *Oct-4* or *Nanog*-promoter-dependent drug resistance is used instead of *Fbx15-neo* for selection of the infected MEFs, the resulting iPS cells exhibit far greater similarity to true ES cells in their molecular signature and functional capacity [37–39]. In other words, one is able to select a population in which reprogramming is more faithful. Specifically, it has been shown that iPS cells derived in this manner have genome-wide gene expression, DNA methylation and histone modification profiles that are almost indistinguishable from normal ES cells [37–39]. These iPS cells also pass the most rigorous functional tests of pluripotency: they can form gametes in chimeric mice [38] and can give rise to all of the tissues of a late-stage embryo formed by tetraploid complementation [39].

It is unclear how these four factors mediate reprogramming. *Oct-4* and *Sox-2* are restricted in expression to pluripotent and multipotent progenitor cells, and both of these transcription factors are essential for ES cell maintenance. Deletion of either gene results in failure to specify the embryonic epiblast, from which ES cells are derived. Oct-4 and Sox-2 may therefore function as activators of pluripotency gene programs in both normal development and reprogramming. However, they are insufficient for reprogramming by themselves [36]. *c-Myc* and *Klf-4* are proto-oncogenes that govern the balance between cellular proliferation and differentiation.

Both are widely distributed in their expression patterns, but are dispensable for pre-implantation embryonic development. So how might these two classes of transcription factors interact to reprogram fibroblasts and generate pluripotent cell lines? ES cells are in a developmental state of ‘suspended animation,’ characterized by tumor-like self-renewal and maintenance of pluripotency. Yamanaka speculates that, as proto-oncogenes, c-Myc and Klf-4 may function to immortalize differentiated cells and disrupt the prevailing program of gene expression [40]. Sox-2 and Oct-4 may then be able to impose a program of pluripotent gene expression on this deregulated genome, resulting in an ES cell phenotype. To test this hypothesis, it would be interesting to see if any additional factors in the same general classes (i.e., proto-oncogenes and pluripotency factors) could substitute for one of the defined factors in this assay. Other functionally equivalent gene products are likely to exist, given that these four factors were culled from an initial screen of only 24 candidate genes. Along these lines, it is notable that the pluripotency factor Nanog was neither required for reprogramming, nor did its overexpression enhance iPS derivation in the defined factor assay [36]. This suggests that reprogramming by cell fusion, and probably also by oocytes, is mediated by different factors and/or mechanisms.

An important question left unanswered by these studies is whether ectopic expression of these four factors is sufficient for the reprogramming event. All four studies used stable retroviral transduction of the factors, which may be resulting in insertional activation or inactivation of other genes important for reprogramming. This possibility is supported by the observation that only an estimated 1% of the cells infected with all four types of virus actually grow out as a reprogrammed clone. It will be interesting to see if reprogramming occurs similarly in repeat experiments where the four factors are overexpressed without genomic insertion. Among other explanations for the observed low efficiency is the possibility that factors must be expressed at precise levels for reprogramming to occur, and that this balance is achieved only in rare cells depending on the site and number of integration events.

Although reprogramming by defined factors is in its early stages, and most would agree ahead of its time, it is interesting to consider some of the challenges remaining before it can be translated to clinical use. First, the technique must be

demonstrated to work in human cells. Anecdotal reports suggest that the same four factors may not be sufficient to reprogram human cells [41]. Second, selection of reprogrammed cells will need to be achieved without using *Oct-4* or *Nanog*-promoter-driven drug-resistance genes in the target cells. If the efficiency can be improved, it may be possible to identify colonies that have been reprogrammed by screening all emerging colonies, without selection, for some signature of pluripotency (e.g., by transcriptional profiling and cell-surface markers). Third, it will be necessary to upregulate the reprogramming factors without retroviral-mediated transduction, given the risks of insertional mutagenesis and unregulated transgene expression. Indeed, tumors attributable to reactivation of the *c-Myc* proto-oncogene were seen in approximately 20% of F1 mice created after germline transmission of iPS cells [38]. Ideally, it would be possible to functionally mimic overexpression of these factors by transient exposure of the target cells to growth factors, drugs or other bioactive molecules such as modulatory RNAs. Although several obstacles remain, the identification of reprogramming factors is a tremendous achievement and has gained universal accolades.

Testis-derived pluripotent cells

All of the methods described above involve reprogramming of differentiated adult tissues to generate a pluripotent cell lines. But what if precursors for pluripotent cell lines already exist in adult tissues? Can such progenitors be identified and used instead of reprogrammed cells? It has long been thought that progenitors of the germ lineage may have pluripotent capacity because they give rise to naturally occurring teratomas. Studies performed several decades ago showed that primordial germ cells (PGCs) isolated from mid-gestation embryos could form experimentally induced teratomas. By culturing PGCs, lines of embryonic germ (EG) cells can be derived that display extensive similarities to ES cells, including the ability to form germline chimeras (reviewed in [42]). Such broad potential in the germ lineage was thought to be lost over the course of embryogenesis, since no similar cell types could be derived from fully developed tissues.

Recent studies suggest, however, that pluripotent cell lines can be derived from the postnatal testis (Figure 1). Shinohara and colleagues demonstrated, in 2004, that after prolonged culture of neonatal testis cells with certain cytokines, rare ES-like colonies unexpectedly

emerged [43]. Cells from these colonies could form teratomas and contribute to germline tissues in chimeras. These lines were called multipotent germline stem (mGS) cells. The cell of origin for these lines was unclear, but the authors speculated that mGS cells arose from spermatogonial stem cells (SSCs). This was a significant claim, given that SSCs were considered to be restricted in potential to the development of male gametes only. Of note, the authors were unable to derive mGS cells from adult testis, which are known to contain SSCs. These results suggested instead that the originating cell might be an embryonic germ cell progenitor such as the PGC, which lingers in the neonatal testis but is lost with age.

Two years later, Guan *et al.* reported that pluripotent cell lines could be derived from adult mouse testis, and more specifically, from SSCs [44]. Using a transgenic strain (*Stra8-GFP*) carrying green fluorescent protein driven by the spermatogonia-specific *Stra8* promoter, they fractionated adult testis cells to enrich SSC and cultured them directly in ES conditions. Colonies expressing surface markers and genes typical of ES cells emerged under these conditions. Cell lines from these colonies were called multipotent adult germline stem (maGS) cells because they could form tissues from all three germ layers in embryoid bodies and teratomas. Remarkably, the authors claimed that the SSCs isolated by *Stra8-GFP* selection were capable of germline transmission when injected directly into blastocysts, without prior culture in ES conditions or derivation as maGS lines. This result was in stark contrast to the results obtained by Shinohara and others, who were unable to demonstrate pluripotentiality of SSCs using similar methods. Guan *et al.* argued that their success was due to subtle but critical differences in culture and enrichment strategies compared with the other studies [44,45].

If derivation of pluripotent cell lines from adult testis is verified, it will provide a means to generate patient-specific tissue progenitors without the difficulties of reprogramming. Clinical use will require the ability to efficiently select germ cell progenitors without genetic markers such as *Stra8-GFP*. Another potential hurdle is that the proper function and specification of tissues derived from mGS or maGS cells may be somewhat limited because both have only 'androgenetic imprints.' Normal development requires the contribution of genes from both maternal and paternal sources [46]. The parental origin of otherwise equivalent gene sequences is encoded

during gametogenesis in the form of differential DNA methylation marks, called imprints. Embryos carrying imprints from only one parental source, even if diploid, cannot develop past the early postimplantation stage. Accordingly, mGS cells could not support the development of live offspring after tetraploid blastocyst complementation, an observation that was attributed to their androgenetic (i.e., entirely male) origin [43]. In chimeras, uniparental ES lines have been shown to be able to contribute to several tissues, including the germline [47,48]. However, androgenetic cell chimeras have been associated with an increase in developmental defects and lethality [49–51], and display a tendency for malignant transformation *in vitro* [52]. It is reassuring that in transplant experiments, Eckardt *et al.* demonstrated that blood stem cells of uniparental origin isolated from mouse fetal liver could reconstitute the entire hematopoietic system of adult recipients [53]. Strain *et al.* have provided a remarkable case report of a patient in whom several tissues, including the hematopoietic system, were found to be of uniparental origin [54]. Therefore, with limitations, uniparental pluripotent cells derived from adult tissues may be able to serve as a source of patient-specific replacement tissues.

Parthenogenesis

Testis-derived pluripotent cells are obviously only obtainable from male patients. Can cells with similar potential be derived from the female germline? Mature mammalian oocytes are typically dormant until they are fertilized. On occasion, oocytes can spontaneously activate and initiate embryonic development, a process known as parthenogenesis [55]. Oocytes can also be activated *in vitro* by brief exposure to ethanol or calcium ionophores. The development of parthenogenetic embryos is limited to the early postimplantation stage due to their uniparental (in this case maternal) origin, as described above. However, pluripotent cell (pES) lines can be derived from parthenogenetic blastocysts (Figure 1) and can contribute to several different tissues, including the germline in chimeric mice [56].

pES lines differ from the previously discussed pluripotent lines in that they are not genetically identical to the oocyte donor, a consequence of chromosome dynamics during oogenesis. After replicating their diploid chromosomes, developing female germ cells undergo two reductive divisions, meiosis I and II. In meiosis I, pairs of homologous chromosomes align and exchange genetic material in a balanced fashion, a process

known as ‘crossing-over.’ The pairs of homologous chromosomes then segregate in the first reductive division. In meiosis II, the centromeres split and the duplicated maternal or paternal chromosomes are separated upon fertilization or activation of the oocyte, to yield a haploid maternal genome. Therefore, in the simplest view, pES lines derived from mature postmeiotic oocytes would be expected to display haplo-identity (sharing one of two alleles) at every locus relative to the oocyte donor. Accordingly, these pES lines would be predicted to be homozygous at the MHC, the main genetic locus determining transplant tolerance. This may be problematic from a therapeutic standpoint since an MHC heterozygous patient may reject her own MHC homozygous pES-derived hematopoietic progenitors due to the lack of one set of her MHC antigens (a phenomenon known as ‘hybrid resistance’ [57]).

Kim *et al.* recently described two strategies for deriving MHC heterozygous pES lines [58]. First, if oocytes are parthenogenetically activated prior to meiosis I while inhibiting maternal and paternal chromosome segregation [59], the resulting pES lines would be expected to be predominantly heterozygous. Indeed, 21 of 23 pES lines derived in this fashion were MHC heterozygous, with the other two being MHC homozygous because of crossing-over. Second, if oocytes are activated before meiosis II while inhibiting chromosome separation, most pES clones would be expected to be homozygous, except those that have undergone crossing-over. Kim *et al.* found that 24 of 72 pES created in this manner had undergone crossing-over to become MHC heterozygous. Notably, pES cell derivation was very efficient, as previously reported, with rates of 37–65% depending on the stage at which oocytes were activated. pES cells created by either method were able to contribute to multiple tissue lineages, shown by *in vitro* differentiation as well as injection into blastocysts to form chimeras. In keeping with their uniparental origin, they were unable to support development of offspring in tetraploid blastocyst complementation. Therefore, while pES cells are not genetically identical to the oocyte donor, their relative ease of derivation and immunological compatibility make their use an attractive candidate for pluripotent cell-based therapy.

Recent work demonstrates the ability to derive pES cells from humans. Revazova *et al.* activated mature human oocytes and generated six parthenogenetic human ES (phES) cell lines from 23 blastocysts [60]. The phES lines were able to

form tissues of all three germ layers by *in vitro* differentiation and teratoma assays. Unexpectedly, all six lines were MHC heterozygous and identical to the oocyte donors. Since the authors used mature oocytes, MHC heterozygosity would be predicted to result from crossing-over in only a fraction of the cases (as described above and in [61]). Nevertheless, the work suggests that pHES lines can be generated with an efficiency and potency similar to mouse pES lines, moving the strategy one step closer to human therapeutic applications.

Summary & future perspective

Which approach is best suited to provide adult tissue-derived pluripotent cells for regenerative therapy? NT yields pluripotent progenitors of the highest capacity, with complete genetic identity to the donor and without introduction of any genetic elements. The technological demands and inefficiency of NT, however, limit the likelihood of its clinical applicability. Unlike NT, the inefficiency of using cell fusion or defined factors for reprogramming can be more easily be overcome by increasing the pool of patient target cells for reprogramming. However, several resultant genetic problems – aneuploidy and/or insertional mutagenesis by selection markers or reprogramming factors – will preclude their use, especially since transgene insertion has caused occasional malignancies in human gene therapy [62]. It will be very difficult to overcome the aneuploidy caused by cell fusion. It is more conceivable that nongenetic strategies will be discovered that can functionally

replace one or more of the defined reprogramming factors. Derivation of pluripotent cells from testis or oocytes avoids many of the technical difficulties of reprogramming. Based on data so far, it seems reasonable to expect that efficiency will be high enough to permit pluripotent cell derivation from the reproductive organs of the patients themselves, without impairing fertility. Whether or not the uniparental cells will allow the generation of a full spectrum of healthy replacement tissues remains an open question.

‘The strategies for generating pluripotent cells from adult tissues have emerged thanks to decades of basic inquiry in developmental biology, providing fundamental knowledge on the nature of pluripotent cells and provoking excitement regarding their potential therapeutic value.’

The strategies described are therefore best viewed as transitional technologies, each with its attendant advantages and limitations for clinical utility (Table 1). All have emerged thanks to decades of basic inquiry in developmental biology, providing fundamental knowledge on the nature of pluripotent cells and provoking excitement regarding their potential therapeutic value. Continued basic and translational research efforts on all of these fronts will ultimately yield the most clinically suitable approach for the derivation of patient-specific cells.

Table 1. Advantages and limitations of current methods for generating pluripotent cell lines.

Method	Advantages	Hurdles/limitations
Nuclear transfer	<ul style="list-style-type: none">• Complete genetic identity with donor• Complete pluripotency <i>in vivo</i> (mouse)	<ul style="list-style-type: none">• Not yet shown to work in humans• Technically difficult and inefficient• Need oocytes or zygotes for reprogramming• Involves embryos
Cell fusion	<ul style="list-style-type: none">• Human pluripotent cell derivation reported [29]• Pluripotency <i>in vitro</i>• No embryos or oocytes involved	<ul style="list-style-type: none">• Inefficient• Aneuploidy• Genetically incompatible to donor
Transduction with defined factors	<ul style="list-style-type: none">• Complete genetic identity with donor• Complete pluripotency <i>in vivo</i> (mouse)• No embryos or oocytes involved	<ul style="list-style-type: none">• Not yet shown to work in humans• Inefficient• Retroviral integration
Derivation from mature testis	<ul style="list-style-type: none">• Relatively efficient• Complete genetic identity with donor• No embryos or oocytes involved	<ul style="list-style-type: none">• No published human report*• Uniparental imprints: limits to pluripotency?
Parthenogenesis	<ul style="list-style-type: none">• Human pluripotent cell derivation reported [60]• Relatively efficient• Genetically compatible (though not identical) to donor	<ul style="list-style-type: none">• Uniparental imprints: limits to pluripotency?• Involves embryos (parthenogenetically created)

*Reference to one unpublished report of human pluripotent cell derivation from testis can be found in [45].

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