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Stem Cell Therapy for Neuromuscular Diseases

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1. Introduction

Neuromuscular disease is a very broad term that encompasses many diseases and aliments that either directly, via intrinsic muscle pathology, or indirectly, via nerve pathology, impair the functioning of the muscles. Neuromuscular diseases affect the muscles and/or their nervous control and lead to problems with movement. Many are genetic; sometimes, an immune system disorder can cause them. As they have no cure, the aim of clinical treatment is to improve symptoms, increase mobility and lengthen life. Some of them affect the anterior horn cell, and are classified as acquired (*e.g.* poliomyelitis) and hereditary (*e.g.* spinal muscular atrophy) diseases. SMA is a genetic disease that attacks nerve cells, called motor neurons, in the spinal cord. As a consequence of the lost of the neurons, muscles weakness becomes to be evident, affecting walking, crawling, breathing, swallowing and head and neck control. Neuropathies affect the peripheral nerve and are divided into demyelinating (*e.g.* leucodystrophies) and axonal (*e.g.* porphyria) diseases. Charcot-Marie-Tooth (CMT) is the most frequent hereditary form among the neuropathies and it's characterized by a wide range of symptoms so that CMT-1a is classified as demyelinating and CMT-2 as axonal (Marchesi & Pareyson, 2010). Defects in neuromuscular junctions cause infantile and non-infantile Botulism and Myasthenia Gravis (MG). MG is a antibody-mediated autoimmune disorder of the neuromuscular junction (NMJ) (Drachman, 1994; Meriggioli & Sanders, 2009). In most cases, it is caused by pathogenic autoantibodies directed towards the skeletal muscle acetylcholine receptor (AChR) (Patrick & Lindstrom, 1973) while in others, non-AChR components of the postsynaptic muscle endplate, such as the muscle-specific receptor tyrosine kinase (MUSK), might serve as targets for the autoimmune attack (Hoch et al., 2001). Although the precise origin of the autoimmune response in MG is not known, genetic predisposition and abnormalities of the thymus gland such as hyperplasia and neoplasia could have an important role in the onset of the disease (Berrih et al., 1984; Roxanis et al., 2001).
Several diseases affect muscles: they are classified as acquired (*e.g.* dermatomyositis and polymyositis) and hereditary (*e.g.* myotonic disorders and myopaties) forms. Among the myopaties, muscular dystrophies are characterized by the primary wasting of skeletal muscle, caused by mutations in the proteins that form the link between the cytoskeleton and the basal lamina (Cossu & Sampaolesi, 2007). Mutations in the dystrophin gene cause severe form of hereditary muscular diseases; the most common are Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD). DMD patients suffer for complete lack of dystrophin that causes progressive degeneration, muscle wasting and death into the

second/third decade of life. Beside, BMD patients show a very mild phenotype, often asymptomatic primarily due to the expression of shorter dystrophin mRNA transcripts that maintain the coding reading frame. DMD patients' muscles show absence of dystrophin and presence of endomysial fibrosis, small fibers rounded and muscle fiber degeneration/regeneration. Untreated, boys with DMD become progressively weak during their childhood and stop ambulation at a mean age of 9 years, later with corticosteroid treatment (12/13 yrs). Proximal weakness affects symmetrically the lower (such as quadriceps and gluteus) before the upper extremities, with progression to the point of wheelchair dependence. Eventually distal lower and then upper limb weakness occurs. Weakness of neck flexors is often present at the beginning, and most patients with DMD have never been able to jump. Wrist and hand muscles are involved later, allowing the patients to keep their autonomy in transfers using a joystick to guide their wheelchair. Musculoskeletal contractures (ankle, knees and hips) and learning difficulties can complicate the clinical expression of the disease. Besides this weakness distribution in the same patient, a deep variability among patients does exist. They could express a mild phenotype, between Becker and Duchenne dystrophy, or a really severe form, with the loss of deambulation at 7-8 years. Confinement to a wheelchair is followed by the development of scoliosis, respiratory failure and cardiomyopathy. In 90% of people death is directly related to chronic respiratory insufficiency (Rideau et al., 1983). The identification and characterization of dystrophin gene led to the development of potential treatments for this disorder (Bertoni, 2008). Even if only corticosteroids were proven to be effective on DMD patient (Hyser and Mendell, 1988), different therapeutic approaches were attempted, as described in detail below (see section 7).

2. Treatment for neuromuscular diseases: gene and cell therapy

The identification and characterization of the genes whose mutations caused the most common neuromuscular diseases led to the development of potential treatments for those disorders. Gene therapy for neuromuscular disorders embraced several concepts, including replacing and repairing a defective gene or modifying or enhancing cellular performance, using gene that is not directly related to the underlying defect (Shavlakadze et al., 2004). As an example, the finding that DMD pathology was caused by mutations in the dystrophin gene allowed the rising of different therapeutic approaches including growth-modulating agents that increase muscle regeneration and delay muscle fibrosis (Tinsley et al., 1998), powerful antisense oligonucleotides with exon-skipping capacity (McClorey et al., 2006), anti-inflammatory or second-messenger signal-modulating agents that affect immune responses (Biggar et al., 2006), agents designed to suppress stop codon mutations (Hamed, 2006). Viral and non-viral vectors were used to deliver the full-length - or restricted versions - of the dystrophin gene into stem cells; alternatively, specific antisense oligonucleotides were designed to mask the putative splicing sites of exons in the mutated region of the primary RNA transcript whose removal would re-establish a correct reading frame. In parallel, the biology of stem cells and their role in regeneration were the subject of intensive and extensive research in many laboratories around the world because of the promise of stem cells as therapeutic agents to regenerate tissues damaged by disease or injury (Fuchs and Segre, 2000; Weissman, 2000). This research constituted a significant part of the rapidly developing field of regenerative biology and medicine, and the combination of gene and cell therapy arose as one of the most suitable possibility to treat degenerative disorders. Several

works were published in which stem cell were genetically modified by ex vivo introduction of corrective genes and then transplanted in donor dystrophic animal models.
Stem cells received much attention because of their potential use in cell-based therapies for human disease such as leukaemia (Owonikoko et al., 2007), Parkinson's disease (Singh et al., 2007), and neuromuscular disorders (Endo, 2007; Nowak and Davies, 2004). The main advantage of stem cells rather than the other cells of the body is that they can replenish their numbers for long periods through cell division and, they can produce a progeny that can differentiate into multiple cell lineages with specific functions (Bertoni, 2008). The candidate stem cell had to be easy to extract, maintaining the capacity of myogenic conversion when transplanted into the host muscle and also the survival and the subsequent migration from the site of injection to the compromise muscles of the body (Price et al., 2007). With the advent of more sensitive markers, stem cell populations suitable for clinical experiments were found to derive from multiple region of the body at various stage of development. Numerous studies showed that the regenerative capacity of stem cells resided in the environmental microniche and its regulation. This way, it could be important to better elucidate the molecular composition – cytokines, growth factors, cell adhesion molecules and extracellular matrix molecules - and interactions of the different microniches that regulate stem cell development (Stocum, 2001).
Several groups published different works concerning adult stem cells such as muscle-derived stem cells (Qu-Petersen et al., 2002), mesoangioblasts (Cossu and Bianco, 2003), blood- (Gavina et al., 2006) and muscle (Benchaouir et al., 2007)-derived CD133+ stem cells. Although some of them are able to migrate through the vasculature (Benchaouir et al., 2007; Galvez et al., 2006; Gavina et al., 2006) and efforts were done to increase their migratory ability (Lafreniere et al., 2006; Torrente et al., 2003a), poor results were obtained.
Embryonic and adult stem cells differ significantly in regard to their differentiation potential and in vitro expansion capability. While adult stem cells constitute a reservoir for tissue regeneration throughout the adult life, they are tissue-specific and possess limited capacity to be expanded ex vivo. Embryonic Stem (ES) cells are derived from the inner cell mass of blastocyst embryos and, by definition, are capable of unlimited in vitro self-renewal and have the ability to differentiate into any cell type of the body (Darabi et al., 2008b). ES cells, together with recently identified iPS cells, are now broadly and extensively studied for their applications in clinical studies.

3 Embryonic Stem Cells (ESCs)

Embryonic stem cells are pluripotent cells derived from the early embryo that are characterized by the ability to proliferate over prolonged periods of culture remaining undifferentiated and maintaining a stable karyotype (Amit and Itskovitz-Eldor, 2002; Carpenter et al., 2003; Hoffman and Carpenter, 2005). They are capable of differentiating into cells present in all 3 embryonic germ layers, namely ectoderm, mesoderm, and endoderm, and are characterized by self-renewal, immortality, and pluripotency (Strulovici et al., 2007).

3.1 Methods for ESCs isolation

hESCs are derived by microsurgical removal of cells from the inner cell mass of a blastocyst stage embryo (Fig. 1). The ES cells can be also obtained from single blastomeres. This technique creates ES cells from a single blastomere directly removed from the embryo

bypassing the ethical issue of embryo destruction (Klimanskaya et al., 2006). Although maintaining the viability of the embryo, it has to be determined whether embryonic stem cell lines derived from a single blastomere that does not compromise the embryo can be considered for clinical studies. Cell Nuclear Transfer (SCNT): Nuclear transfer, also referred to as nuclear cloning, denotes the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a cloned embryo (Wilmut et al., 2002).

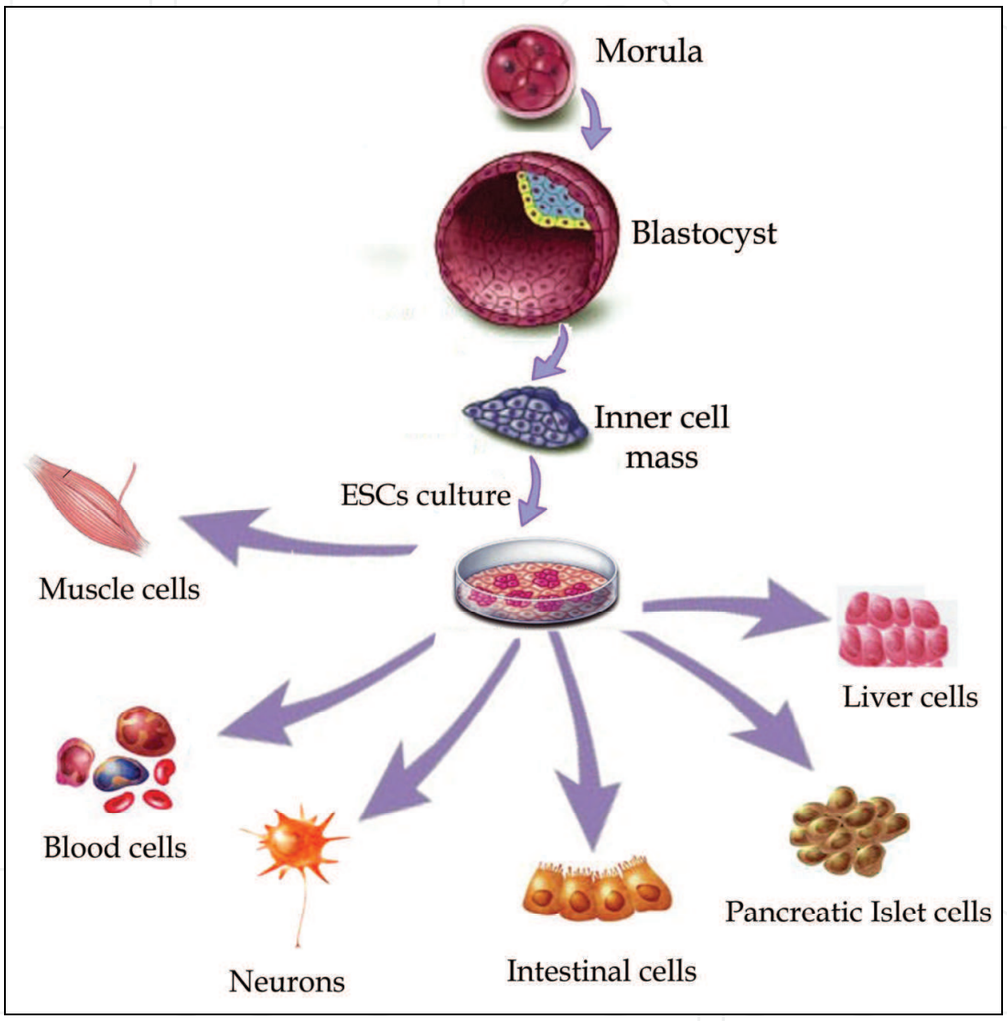

Fig. 1. ESCs differentiation. Differentiation potentiality of human embryonic stem cell lines. Human embryonic stem cell pluripotency is evaluated by the ability of the cells to differentiate into different cell types.

3.1.1 ES cells from single blastomeres

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3.1.2 Cell Nuclear Transfer (SCNT)

Nuclear transfer, also referred to as nuclear cloning, denotes the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a cloned embryo (Wilmut et al., 2002). The first application of this technique was in 1996 the creation of Dolly the sheep (Campbell et al., 1996). Transferred to the uterus of a female recipient, this embryo has the potential to grow into a clone of the adult donor cell, a process termed "reproductive cloning." When explanted in culture, this embryo can give rise to embryonic stem cells that have the potential to become any or almost any type of cell present in the adult body. This process is also called "nuclear transplantation therapy" or "therapeutic cloning" because embryonic stem cells derived by nuclear transfer are genetically identical to the donor and thus potentially useful for therapeutic applications. It might substantially improve the treatment of neurodegenerative diseases, blood disorders, or diabetes, whose therapies are currently limited by the availability or immunocompatibility of tissue transplants (Hochedlinger and Jaenisch, 2003). Unfortunately, reproductive cloning is a largely inefficient and error-prone process that results in the failure of most clones during development due both to activation of inadequate pathways of early embryonic development (Solter, 2000) and suppression of pathway of differentiation (Rideout et al., 2001). In contrast, reprogramming errors do not appear to interfere with therapeutic cloning, because the process appears to select for functional cells. Recent advances in the field of nuclear cloning showed that most clones die early in gestation while cloned animals share abnormalities regardless of the type of donor cell or the species used, correlating with aberrant gene expression (Hochedlinger and Jaenisch, 2003). Although experiments in animals showed that by SCNT it is possible to obtain primate ES cells (Byrne et al., 2007) and nuclear cloning combined with gene and cell therapy represents a valid strategy for treating genetic disorders (Rideout et al., 2002), the low efficiency of the technique, the difficulties in obtaining human eggs and the arising ethical problems are significant challenges to the widespread use of SCNT for the production of hESC.

3.2 Characterization of ESCs

Human embryonic stem cells (hESCs) were first derived from the inner cell mass (ICM) of the blastocyst stage (100–200 cells) of embryos generated by *in vitro* fertilization (Thomson et al., 1998), but methods have been developed to derive hESCs from the late morula stage (30–40 cells) (Strelchenko et al., 2004), from arrested embryos (16–24 cells incapable of further development) (Zhang et al., 2006) and single blastomeres isolated from 8-cell embryos (Klimanskaya et al., 2006). Because hESCs have the potential to differentiate into normal tissues of all types, the ability to derive and maintain hESCs in culture gave rise to the possibility of having an unlimited supply of normal differentiated cells to engineer diseased tissues to regain normal function (Moon et al., 2006; Skottman et al., 2006).

Nowadays, several studies demonstrating hESC differentiation into specific cell lineages use feeder layers of heterologous cells to maintain hESCs in culture and to signal the hESCs to differentiate into specific cell types (Conrad et al., 2008; Takahashi and Yamanaka, 2006). After transplantation into the recipient, the hESCs and their progeny could be exogenously controlled if they differentiated into malignant cells or if they otherwise grew and/or functioned in an unwanted lineage; if hESCs are to be useful in generating normal tissues for the treatment of human disease, the tissues to be transplanted must be compatible with the host such that the cells derived from the hESCs will not be recognized as "foreign" and rejected as would any transplanted tissue from an unrelated donor.

The first human stem cell line bank was opened in 2004 in the United Kingdom (http://www.ukstemcellbank.org.uk/). The National Institutes of Health registry (http://stemcells.nih.gov/research/registry/) has also archived a number of hESC lines and established criteria for demonstration of the pluripotency of these lines. Cells should be able to give rise to any cell lineage of the body and thus to form a teratoma (a tumour containing tissues from the 3 primary germ layers) after injection in an immune-suppressed animal and should be capable of unlimited self-renewal. A number of scientific/medical issues need to be addressed before stem cells can be considered safe for clinical applications. In fact for hESCs to be useful for therapy, technologies must be developed to provide them with the specific signals required to differentiate in a controlled lineage, to regulate and/or shut down the growth of hESCs and their progeny once they have been transferred to the recipient. Pluripotency is evidenced by the ability to form teratomas when transplanted in immunodeficient mice, the concern exists that these cells could form malignant tumours in their new host. One strategy for dealing with this problem is to select pure populations of more committed cells for transfer. Demonstrating genetic and epigenetic stability will therefore be important before these cells are used clinically. Moreover attention has to be focused on circumventing the host rejection of transplanted, non-autologous hESC-derived cells (Strulovici et al., 2007).
Therefore, karyotypic abnormalities have been described in several hESC lines, although changes might be at least partially dependent on culture techniques (Mitalipova et al., 2005). In additional to biologic issues directly affecting the stem cell product, it is fundamental that controlled, standardized practices and procedures be followed to maintain the integrity, uniformity, of the human stem cell preparations. Because of stem cells are maintained and expanded in vitro before transplantation, culture conditions compatible with human administration must be used. Feeder cells and sera of animal origin have to be reduced and ideally avoided to reduce the potential risk of contamination by xenogeneic protein. Consequently, life-long immunosuppressive therapy, which can lead to infections and organ-based toxic side effects, such as nephropathy, might be required to prevent graft rejection (Brignier and Gewirtz, 2010).

3.2.1 Expression markers

Cell origins are often defined by one or more cell surface and or intracellular epitopes unique to that particular cell type. Stage-specific embryonic antigen (SSEA) markers are used to distinguish early stages of cell development, denoting pluripotency. These markers are globo-series glycolipids and are recognised by monoclonal antibodies. The SSEA-4 epitope is the globo-series glycolipid GL7. It has been demonstrated that GL7 can react with antibodies to both SSEA-3 and SSEA-4 (Kannagi et al., 1983a; Kannagi et al., 1983b). Human ES cells will express SSEA-3 and -4 during pluripotency and only SSEA-1 upon differentiation (Andrews et al., 1996; Reubinoff et al., 2001; Thomson et al., 1998; Thomson and Marshall, 1998). The TRA-1-60 epitope adheres to a particular epitope of the proteoglycan and is sialidase sensitive, whereas antibody TRA-1-81 reacts with another unknown epitope of the same core proteoglycan molecule. Nanog is a NK-2-type homeodomain gene thought to encode a transcription factor that is critically involved in the self-renewal of stem cells. Thus, it may possibly act to repress genes necessary for differentiation and activate those involved in self-renewal. Lin and colleagues (Lin et al., 2005) demonstrated that the tumour suppressor p53 binds to the promoter of Nanog.

Therefore, p53 can stimulate p53-dependent cell-cycle arrest and apoptosis when genetic integrity is not preserved. Oct-4, a POU-domain transcription factor, is highly expressed in ES cells (Niwa, 2001; Reubinoff et al., 2001; Thomson et al., 1998) and has been shown to be essential for maintaining pluripotency (Niwa, 2001). It has been reported that Oct-4 transcripts are nearly exclusively found in pluripotent cells *in vivo* and within culture. Oct-4 down-regulation is observed in differentiating cells (Rosner et al., 1990). Not only is Oct-4 necessary for the maintenance of pluripotency, but its expression level governs three cell fates once differentiation occurs (Hay et al., 2004; Niwa et al., 2000). Several candidate genes have been reported as targets of Oct-4 based on stem cell expression patterns and immunoprecipitation, but few have been conclusively verified. Target genes of Oct4 include Rex-1, Lefty-1, PDGFalfaR and Utf-1, and those cooperating with Oct4 include Sox2. In the ongoing search for the identification of pluripotent markers, Xu and colleagues have reported that the catalytic component of telomerase, telomerase reverse transcriptase or hTERT, is expressed in undifferentiated cells and down-regulated upon differentiation (Xu et al., 2001).

3.3 Potentialities of ESCs

ES-derived progenitors possess excellent self-renewal and regenerative potential, but the research on these cells is at the beginning. Recently, Jaenisch and collaborators published that the adult cells contain unipotent and multipotent stem cells such as haematopoietic stem cells even if totipotent and pluripotent cells are restricted to the early embryo (Jaenisch and Young, 2008). Although the decrease in developmental potential, the nuclei of most of adult cells maintain nuclear plasticity to reset to an embryonic state. It's possible to enhance this process by exposing the oocyte to specific factors through nuclear transfer or the cells to pluripotent cell-specific factors by driving over-expression of defined transcription factors. However before the clinical applications of these cells, it's needed to optimize the engraftment of hESCs and the development of a protocol to obtain similar populations of muscle precursors from human ES cells. ESCs are derived rather easily, and they can grow indefinitely in culture. Second, embryonic stem cells can be manipulated genetically by homologous recombination to correct a genetic defect (Rideout et al., 2001). Recently, Jiang and collaborators demonstrated pluripotency of mesenchymal stem cells derived from adult marrow, differentiating into cells of all three germ layers both in vitro and in vivo after being injected into blastocysts (Jiang et al., 2002). Unfortunately, they did not assess the ability of these cells in correcting a disease phenotype into both human or mouse animal models. Embryonic stem cells can become any type of cell through the use of specific culture conditions or genetic manipulation. To avoid the ethical and practical limitations of therapeutic cloning mentioned above, it would be useful to reprogram somatic cells directly into embryonic stem cells without the use of oocytes. For this reason, it's necessary to deeply understand the role of several molecular factors in establishing and maintaining pluripotency, such as Oct-4 (Niwa et al., 2002). Oct-4 null embryos cannot form a pluripotent inner cell mass, consequently their development is arrested. To circumvent the need for human oocytes, it could be possible to modify the expression of Oct-4 and its related genes in somatic cells to reprogram their nuclei to an embryonic state.

3.4 Drawbacks to clinic use of ESCs

3.4.1 Host response to grafted tissue

It is probable that ESCs will suffer from the same acute and chronic rejection problems that accompany other grafts and it is likely that this question will not be answered until these

cells are implanted into humans for the first time. Three methods have been proposed to avoid this problem. The first requires the use of somatic cell nuclear transfer (SCNT) techniques, as used to clone animals, to personalize ESCs. The nucleus of a somatic cell from the individual to be treated would be transferred into an enucleated donor oocyte, which would then be used to derive a blastocyst and subsequently isolate ESCs lines that would be genetically identical to the patient (Yang et al., 2007). In this case, any cell generated from the personalized ESC line should not be rejected. However, despite the claims of the South Korean Dr Hwang Woo-suk in 2005 that have subsequently been shown to be fraudulent, SCNT has not been successfully performed in human oocytes. It appears that the process is more complicated in humans than in animals where this technology has been used successfully in many species. The second method makes use of the capacity of ESCs to differentiate into multiple tissues and would involve replacing the recipients immune system with haematopoietic cells generated from the same ESC line as that used for tissue replacement. This technique has been used in solid organ transplants where patients have previously received bone marrow transplantation, and these patients did not require immune suppression (Helg et al., 1994). The third method used genetic manipulation to engineer ESCs in which MHC molecules or other immune effectors have been deleted (Hyslop et al., 2005). All these methods are under development. In addition to the immune response to the cells themselves, animal products are used to isolate ESCs in every methods, and it cause expression of animal proteins on the surface of the ES cells. This will also induce an immune response and a large amount of work is currently going into deriving and maintaining ESCs in total absence of these animal products and also undertaking these processes to the good manufacturing practice (GMP) standards required for clinical use. It is in the development of GMP protocols for the derivation and manipulation of ES cells.

3.4.2 Cell proliferation and differentiation

In addition to the problems connected with immune interactions, there are other important problems in providing a suitable number of cells for transplantation. The first problem is related to have the very large numbers of cells required for tissue replacement without tumour formation. As previously discussed, ES cells can form teratomas and therefore all undifferentiated cells will need to be removed from a graft. It is possible to circumvent this problem genetically modifying the cells by a suicide gene system. The expression of the suicide gene could be driven by the promoter of a gene such as Oct4 that is only expressed in undifferentiated cells; this technology has been used in gene therapy systems. Activation of the suicide gene by drug treatment of cell cultures would give rise to the death of the undifferentiated cells not affecting differentiating cells. Other methods include the expression of a genetic label such as the green fluorescent protein or a marker that enables cell sorting by FACS (Strulovici et al., 2007). In this case, cells could be selected using FACS for the surface markers expressed either excluding undifferentiated cells or positively selecting for the required differentiated cell type. Even if contaminating undifferentiated cells are removed, remain the problem due to the necessity to have a good number of mature cells able to form a robust graft and also maintaining the cells where they have to perform their therapeutically action. Differentiation protocols are being studying to generate very large number of cells (Joannides et al., 2007) and scaffolds able to keep the cells in place (Ferreira et al., 2007). Another aspect of tissue replacement that cannot be ignored is that few tissues are formed from a single uniform cell type. In order to regenerate a functional organ, it will be necessary to develop other structures such as vasculature and lymphatic drainage

systems as well as complex mixed cell populations (Caspi et al., 2007). These problems present a major challenge to tissue culture technology not only for ESCs but also for adult stem cells, and the development of new systems will be important in order to utilize ES cells to their maximum potential.

3.4.3 Ethical implications to clinical use of ESCs

The use of hESCs in medical research has focused much attention from many sectors of the public. Religious, historical, cultural, medical, and other points of view have contributed to a very vigorous and wide-ranging discourse over the use of these materials (Leist et al., 2008). Some consider research with hESCs to be inherently immoral because these individual's believe that life begins with fertilization of the ovum, and the destruction of an embryo with the potential to develop into a viable human being is thought like an infanticide. For this reason, the American federal government severely restricted access and use of hESCs in 2001. These restrictions have now been largely overturned by the Obama administration. In contrast, proponents of this line of research insist that the potential benefits to human from this research mitigate such concerns. They also argue that hESCs are made from unwanted fertilized ovum that would likely be destroyed in any event. Stem cells created by means of nuclear transfer share the same ethical concerns. Furthermore, because these cells have the potential to generate a complete embryo, they also raise the even more highly charged possibility of cloning human beings, so-called reproductive cloning (Brignier and Gewirtz, 2010). Many organizations and countries have already banned reproductive cloning of human beings. Because this procedure can be used to generate stem cells for therapeutic purposes, in countries where this type of cloning is legal, such as Australia and the United Kingdom, the created embryos must be destroyed within 14 days. A human nucleus is transferred into an animal's oocyte, creating a hybrid embryo that must be destroyed within 2 weeks and cannot be implanted. Clearly, creation of such tissues raises even more complex issues. Finally, the issue of financial compensation for embryo and gamete donors is also controversial, with guidelines for this problem being proposed by the International Society of Stem Cell Research (http://www.isscr.org/guidelines /index.htm). Everyone involved in the debate want very much to avoid the development of an underground black market in spare embryos ((Brignier and Gewirtz, 2010).

3.4.4 Clinical applications of ESCs

Among the important potential applications of gene therapy to hESCs is the correction of genetic diseases. Although many hereditary disorders can be targeted by gene therapy vectors alone, the combination of gene therapy and stem cell therapy may have added utility, where cells differentiated from hESCs would act as factories to produce therapeutic proteins or where a high proportion of corrected cells could be developed. In the case of circulating proteins (*e.g.*, factor IX, factor VIII, von Willebrand factor, *a*1-antitrypsin), it may be possible to establish tissue reservoirs distant from the normal site of the secreted product (Mountford, 2008). The cells differentiated from hESCs can be delivered to a site accessible and receptive to transplantation, even if that tissue is not the normal site of production of the protein of interest. When the product is not secreted, the hESCs with their regulated genetic characteristics must be differentiated to the correct cell type (*e.g.*, the cystic fibrosis transmembrane conductance regulator for cystic fibrosis or dystrophin for muscular dystrophy). Such proteins can exert their influence only at the appropriate site, and there is no known mechanism by which cells expressing the protein remote from the affected tissue

could have a therapeutic effect. Additional drawbacks are to be solved in order to obtain the successful therapeutic application of gene-modified hESCs including whether the hESC themselves, or the expressed product, will be toxic or immunogenic in the recipient. If the recipient of the cells, never exposed to the protein before, as in deletion or nonsense mutants, can be showed an immune reaction against the protein, limiting the effectiveness of the therapy (Mountford, 2008).

To develop hESC-based therapies, it is obvious that strategies capable of mitigating risks related to the therapeutic use of hESCs should be pursued the development of the therapies. These strategies might include several non–mutually exclusive mechanism for ablating all genetically modified cells while sparing most endogenous cells. The introduction of a step in the development of the therapy at which a single genetically modified cell would be isolated, expanded, and characterized with respect to the location of the mutation would allow an analysis of the relative risk of the insertion site. Similar limiting dilution cloning strategies are now routinely performed during the original isolation of a stem cell line to ensure that only one karyotype is represented (Mountford, 2008). Progress in understanding how insertional mutagenesis can lead to uncontrolled growth of stem cells is an essential prerequisite for this analysis and is currently an active area of research. Genetic modification can be used to enhance our ability to conduct such an isolation step by adding a convenient ligand for cell isolation. Genetic modification is also potentially useful for solving the problem of uncontrolled cell growth. Incorporating the genes for an ablation strategy at the same time as the genes for the therapeutic strategy would give the best chance of ensuring that the safety mechanism will be present when and if needed. Initial applications of genetically modified hESCs are likely to occur where the risk/benefit ratio tilts in favour of benefit, as in fatal disorders for which there is no therapy. The risks of the hESC therapies will have to be understood and probably reduced to maintain an appropriate risk/benefit ratio before these technologies can be applied to diseases that are inherently less dangerous to the patient. Gene therapy should prove to be valuable in reducing the risks associated with making hESC therapy a reality (Mountford, 2008).

In particular, the ability to generate cells with in vivo muscle regenerative potential in culture and systemically transfer them to recipients is an important step towards the therapeutic application of ES cell-derived cells. Unfortunately, it's not still provided a reproducible method to generate ES-derived myogenic progenitors for skeletal muscle regeneration. An ES cell–based therapy would have many advantages: it could allow the transplantation of a more primitive cell with greater replicative potential and patient-specific ES cells could be induced from adult somatic cells. Moreover, the derivation of an ES cell–derived myogenic population with proliferative and regenerative potential has not been accomplished. Only two papers described some evidences for engraftment on transplantation of an ES cell–derived population but they were limited to qualitative detection of donor derived cells in recipient muscle (Bhagavati and Xu, 2005; Kamochi et al., 2006). The Pax3 ES cell–derived population exhibited good potential for skeletal muscle regeneration but several studies concerning their capacity of replenish satellite cells-niche are in progress (Darabi et al., 2008a). On the other hand there is enough optimism about the ESC-based therapies because of it may offer reliable and cost-effective therapeutic substitute for treatment of neuromuscular disorders as DMD or BMD. Moreover there are critical issues that need attention in case of ESC-based approaches. No enough knowledge there are about genetic and epigenetic stability of hESC lines over longer time periods and it's not negligible the possible uncontrolled cell proliferation and reprogramming of ESCs in vitro.

The injection of these cells could probably generate an immune rejection needed an immunosuppressive therapy. Optimization of a generic differentiation protocol and its empirical testing with a better understanding of the molecular processes governing ESC differentiation can guarantee the clinical use of these cells. In conclusion the success of the clinical application of adult or embryonic stem cells will be employed to a large-scale production of desired cell type with appropriate functionality, an optimal number of cells for transplant, a modification of less invasive delivery systems and a technique to label cells for transplant and subsequent tracking of cell fate.

4. Induced Pluripotent Stem Cells (iPS)

The major impediment to ES based therapies in humans involves the moral and ethical problems linked to the blastocyst destruction and oocyte donation necessary to generate patient-specific pluripotent stem cell lines. These limitations have encouraged researchers to understand the mechanisms regulating pluripotency and to experimentally determine its gene expression program. Recent works describe the derivation of ES-like iPS cells from adult mouse and human cells (Nakagawa et al., 2008; Takahashi et al., 2007; Yu et al., 2009) by introducing specific sets of genes encoding transcription factors expressed in undifferentiated ES cells to reprogram the adult cells. Although initial studies indicating these cells to share characteristics of ''true'' ES cells, more detailed work is needed to determine how closely they resemble ES cells. Like ESCs, iPS cells can differentiate into all adult cell types. Researchers now have the ability to create tissue-based models of human disease based on cells derived from individual patients. This technology has the potential to herald a new era of patient-specific, cell-based medicine; however, given the oncogenic potential of undifferentiated iPS cells due to the unsafe reintroduction of these genes (Takahashi and Yamanaka, 2006), the safety of these cells has to be tested accurately before attempting any therapies. It has been demonstrated that continuous over-expression of transcription factors, especially the c-myc oncogene, may be associated with tumorigenesis (Takahashi et al., 2007). Even if it was demonstrated that the promoters of these viral vectors can be silenced by endogenous gene expression during reprogramming, chimeric mice derived from iPS cells were showed to be more prone to tumour formation. Following ameliorations in iPS technology, Nakagawa and co-workers generated pluripotent stem cells without c-myc over-expression both from mouse and human fibroblasts, with lower efficiency (Nakagawa et al., 2008). In addition, chimeric mice created from these non-myc iPS cells do not form tumours at an elevated rate. Recently, Chuang et al. proposed the use of baculoviral systems as a new gene delivery vector for stem cell engineering, and in particular for transgenic expression in human ESCs (Chuang et al., 2007). These vectors can be used for large segments, more than 30 kb, that do not fit into adenoviral or lentiviral vectors and could limit the risk derived from the great immunogenicity of adenoviral vectors.

4.1 Methods for reprogramming human fibroblasts into iPS cells

4.1.1 Production of iPS cells using viral integration

In 2006, Yamanaka et al. identified four transcription factors—Klf4, Sox2, Oct4, and c-Myc able to transform mouse fibroblasts in pluripotent clones through retroviral transduction. The clones were selected for their ability to reactivate the non essential Oct4 downstream target gene, Fbx15 (Takahashi and Yamanaka, 2006). This first-generation of

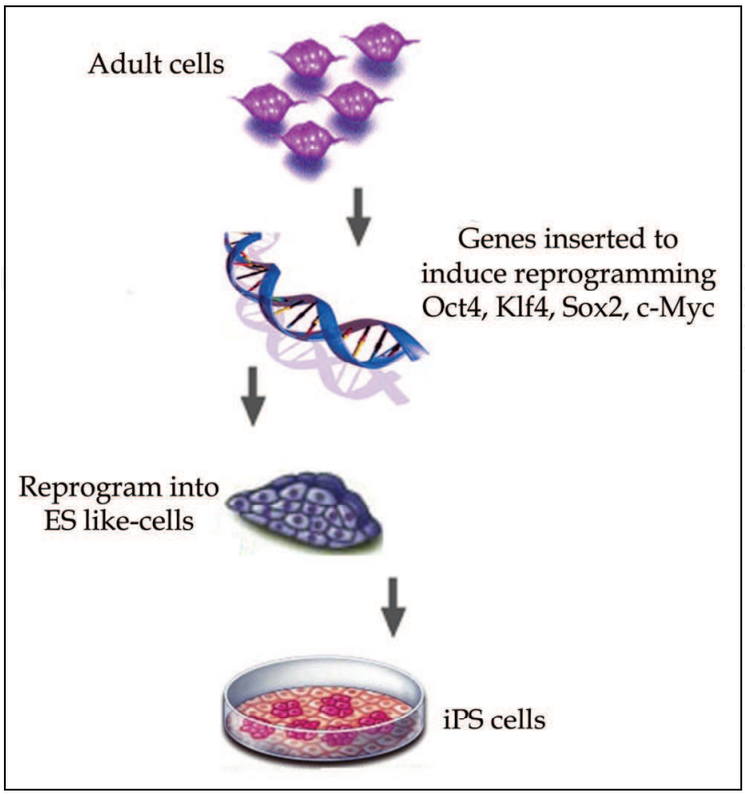

Fig. 2 Generation of iPS cells. Reprogramming of adult stem cells in iPS cells mediated by Oct-4, Klf4, Sox2 and c-Myc give raise to ES like cells with embryonic potential.

iPS cells exhibited partial demethylation and reactivation of the canonical pluripotency governing genes Oct4 and Nanog. In 2007, fully pluripotent iPS cells were generated by increasing the stringency of the selection strategy and selecting for reactivation of the pluripotency regulators Oct4 and Nanog themselves (Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007). These new second-generation iPS cells had fully demethylated Oct4 and Nanog promoters. These cells could not form viable mice by tetraploid complementation; an assay in which ES or iPS cells are injected into tetraploid blastocysts, resulting in embryos derived entirely from the injected cells, while extraembryonic tissues are derived from the host blastocyst. It is controversial if this failure is due to effects of the randomly integrated retroviral vectors used for reprogramming or represents a more fundamental defect in the developmental potency of iPS cells. Pluripotent iPS cells could be identified on the basis of their morphology, eliminating the need for genetically modified reporter genes and permitting the isolation of iPS cells derived from human fibroblasts (Park et al., 2008; Takahashi et al., 2007; Yu et al., 2009). After the work of Yamanaka, Yu et al generated iPS cells starting from a combination of Oct4, Sox2, Nanog, and LIN28. The finding that direct epigenetic reprogramming with different combinations of transcription factors can be applied to human cells represents the breaking of a species barrier that SCNT has yet to overcome. The therapeutic value of iPS cells is the presence of proviral integrations harboring known oncogenes, particularly c-Myc, as well as Oct4 and Klf4. c-Myc was dispensable for iPS generation from fibroblast target cells (although iPS formation occurred with reduced efficiency), and chimeric mice derived from three-factor iPS cells (Oct4, Sox2,

and Klf4) did not exhibit tumour formation while the cells derived from the first four-factors showed tumorigenic characteristics (Nakagawa et al., 2008; Wernig et al., 2007). Despite the non essential role of c-Myc in the reprogramming process, the potential for insertional mutagenesis and the oncogenic properties of the other reprogramming factors has prompted several groups to undertake direct epigenetic reprogramming approaches using either nonviral methods, or retrospectively eliminate proviral integrations after iPS cell generation.

4.1.2 Production of iPS cells without viral integration

Different reasons have drive the researchers to evolve strategies for lentivirus-free iPS cell generation. First, the introduction of foreign DNA able to integrate in random positions in the genome represent a risk for cell physiology giving rise to an insertional mutagenesis. The foreign DNA could destroy reading frames of genes or influences gene regulation. Moreover silencing of lentiviral transgenes is incomplete in iPS cells leading to reactivation of lentiviral vectors (Wernig et al., 2007). In fact basal expression of lentiviral reprogramming factors is found even in fully reprogrammed cells (Hotta and Ellis, 2008; Pfannkuche et al.). Pfannkuche et al. demonstrated that in murine iPS cultures, there was different expression of viral Oct4 by three orders of magnitude between spontaneously differentiating iPS and stable subclones of the same origin (Pfannkuche et al.). From several studies, it is known that altered levels of pluripotency factors influence the fate of pluripotent cells. Overexpression of Oct4 seems to direct ES cells towards an endodermal fate (Niwa et al., 2000; Pesce and Scholer, 2001). More strikingly, elevated levels of Sox2 can alter the whole network of pluripotency factors and abolish maintenance of pluripotency (Kopp et al., 2008; Rizzino, 2009). The best strategy to eliminate reprogramming factors or to prevent integration of foreign transgenes for generation of iPS cells for therapeutic issues could be to employ techniques that are able to reprogram somatic cells without the use of any type of DNA. One possibility to achieve this aim is the use of cell-permeable transcription factors. The application of cell permeable proteins for direct reprogramming has been shown with human newborn fibroblasts, but, again, the efficiency of reprogramming appears low (Kim et al., 2009a). Kim and co-workers includes incubation with the cell permeable factors for 42 days with six passage steps during incubation. Even if the efficiency appears low, this study demonstrated for the first time that cell-permeable proteins can reprogram somatic cells. However, the long incubation time of 6 weeks in the presence of recombinant reprogramming factors in culture media, could be difficult. The application of cell-permeable reprogramming factors is a very promising approach that certainly justifies further investigation to achieve efficient, less time consuming reprogramming of cells from adult donors. Recent results suggest that a substantial amount of cell permeable reprogramming factors is enclosed in endosomes after cell uptake and might therefore be unavailable for reprogramming (Pan et al.). Another possibility to perform reprogramming is the introduction of RNA that can be transfected into cells. Transport of synthetic mRNAs to the nucleus is not necessary and, therefore, the transfection efficiency to deliver mRNA to the cytoplasm might be high. The stability of RNA limit the reprogramming efficiency and to obtain the right amount of protein translated it will be necessary several rounds of transfection to achieve reprogramming. A further strategy to induce pluripotency without the generation of stably transfected cells is the use of non integrating viruses. Adenoviral vectors were designed to express reprogramming factors and transport the coding sequence transiently into the target cells.

Application of the adenoviral approach on fetal human fibroblasts resulted in the generation of stable human iPS cells without viral integration. The human iPS cells form teratomas *in vivo*, which is the most stringent assay applicable to test their pluripotency (Zhou and Freed, 2009). Adenoviral vectors could enhance the production of virus- free iPS cells but the use of viruses that are DNA-based still bears a residual risk of integration into the host genome. This might be overcome with an RNA-based virus to generate safe iPS cells without application of DNA based vectors.

Another possible approach is the use of a single plasmid containing Oct4, Klf4, c-myc and Sox2 stably transfected into somatic cells for reprogramming. The plasmid expressed a single mRNA that codes for all four reprogramming factors and the red-fluorescent protein (RFP) in order to discriminate the transfected cells (Kaji et al., 2009). Different studies are also focused on the application of transposon-based systems. The application of the piggy-BAC transposon allows delivery of reprogramming factors into cells of different organisms and stable integration of the reprogramming genes into the genome by action of the transposase enzyme transiently transfected in a plasmid, and it is able to catalyse the integration of the reprogramming factors (Woltjen et al., 2009). The whole cassette is flanked by inverted terminal repeats, which mediate insertion into the genome by the host-factor independent activity of a transposase. The principal advantage of the system is not only the higher efficiency to deliver reprogramming factors but also the possibility to remove the transgene. Expression of reprogramming factors from the transposon was controlled by a doxicyclin inducible promoter, in order to guarantee a temporal control of transcription factors.

4.1.3 Generation of iPS cells with less than Yamanaka's four factors

Researchers are looking for solutions to reduce the number of factors needed for reprogramming. One of the reasons is to reprogram the cells using chemical compounds in order to standardize the procedure conditions, to control the response of each compound and to regulate the reprogramming factor expression. Another reason is that reduction of reprogramming factors to perhaps a single transgene could guarantee the understanding of mechanisms underlying reprogramming. Between the four factors used by Yamanaka, c-myc has been thought to have the highest oncogenic potential. The three other factors were not associated with tumorigenesis. The role of Klf4 in carcinogenesis is ambiguous. It can act as a tumour suppressor gene, especially in gastrointestinal tumours, or as oncogene, in the development of breast carcinoma where it is involved in the early phase of malignant transformation (Rowland and Peeper, 2006). The first reduction to three factors Oct4, Sox2 and Klf4 induced pluripotency in mouse and human fibroblasts (Nakagawa et al., 2008; Wernig et al., 2007) and other somatic cell types (Park et al., 2008). Moreover the generation of iPS cells was possible combinating Oct4, Sox2 and c-myc in the absence of Klf4. It was concluded that Oct4 and Sox2 in combination with either c-myc or Klf4 were sufficient (Park et al., 2008). Other researchers studied the use of another set of factors including Sox2, Oct4 and the nuclear receptor Essrb, which could replace Klf4 for reprogramming (Feng et al., 2009). However, the estimated reprogramming efficiency was ten times higher than in the original 4-factor approach in fibroblasts. Finally, reprogramming using only Oct4 in the absence of any other factor became possible in neural stem cells derived from adult mice (Kim et al., 2009b) and humans (Kim et al., 2009a). Another strategy is the substitution of single reprogramming factors by small molecules. For example c-myc or Klf4, could be substitute with valproic acid (VPA), thus making reprogramming of human fibroblasts

possible only by retroviral transduction of Oct4 and Sox2 (Huangfu et al., 2008), with a 200-fold reduction of reprogramming efficiency compared to Oct4, Sox2 and Klf4 in combination with VPA. VPA acts as histone deacetylase inhibitor (Huangfu et al., 2008). Other authors utilized high-throughput assays to identify chemical factors which can substitute reprogramming genes. For example, Klf4 could be chemically substituted by the application of kenpaullone (Lyssiotis et al., 2009), and two other small molecules (BIX-01294 and Bayk8644) could enable reprogramming of embryonic fibroblasts with only Oct4 and Klf4.

4.1.4 Enhancement of iPS production

Due to the low efficiency of iPS production, different factors have been investigated in order to improve this procedure. Simultaneous transduction of c-myc, Oct4, Sox, Klf4, with Nanog and Lin28 increases efficiency dramatically (Liao et al., 2008). Other studies have shown that expression of p53 siRNA and UTF1 dramatically increases iPS colonies (Zhao et al., 2008). While p53 siRNA remarkably increases the number of iPS colonies, UTF1, a pluripotent marker and necessary for the maintenance of pluripotency in mES cells (Gaspar-Maia et al., 2009). By affecting different pathways, these two factors together can synergize iPS generation (Zhao et al., 2008). Other pathways which have an impact on iPS cells formation include the TGFβ and the MEK-ERK pathway. The role of these pathways in cell survival has made them beneficial in reprogramming studies. Chemical inhibitors of the MEK pathway apparently inhibit the growth of non-iPS cells while increasing the growth rate in reprogrammed iPS cells. Suppressing MEK and TGFβ pathways concurrently with chemical compounds increases iPS amounts. This study showed that combined suppression of both pathways resulted in extensive amounts of iPS generation in comparison to individual inhibition of each pathway (Lin et al., 2009). Repression of the Ink4/Arf locus which is a regulator of the p53 p21 pathway has a positive effect on reprogramming. Different pluripotency genes are controlled by the expression of different micro RNAs expressed in the cell. For example, let-7 is one of the miRNAs which targets the 3'UTR and ORF's of several pluripotency expressed genes including c-myc, Oct4, Sox2 and Nanog. Suppressing let-7 by antisense inhibitors improves iPS generation several fold in mouse embryonic fibroblasts (MEFs) (Melton et al. , 2010). Reduced oxygen levels favour the growth of haematopoietic stem cells and maintain ES cells in a pluripotent state. Induction of pluripotency in hypoxic conditions increases the number of iPS colonies and vitamin C elevates iPS reprogramming efficiency (Esteban et al. , 2010). The increase in iPS formation is not due to its antioxidant activity but vitamin C inhibits p53, thus facilitating the induction of iPS cells (Shi et al. , 2010). Vitamin C extends the life span of both iPS and MEF cells pointing at roles for vitamin C in anti-ageing. Composition of the iPS culture medium also defines the efficiency of iPS formation. The use of transcription factors together with different chemical agents can enhance iPS induction. This can result in the derivation of more pluripotent cells in a shorter time, making its application more convenient for clinical purposes in a not too far future.

4.2 Clinical applications of iPS

Following the success of haematopoietic stem cell therapy in the treatment of haematological diseases, the potential application of cell based therapy has been extended to the treatment of other human diseases. In particular, different types of adult stem cells, including bone marrow, peripheral haematopoietic, and mesenchymal stem cells (MSC) have been evaluated in the treatment of different diseases (Charwat et al., 2010; Siu et al.,

2010). ESCs have been explored for tissue regeneration because of their ability to differentiate into various therapeutic relevant cell types *in vitro* (Murry and Keller, 2008). Despite this, there is limited progress in the use of ESCs for tissue regeneration in humans due to various technical, social and religious issues (Kiskinis and Eggan). The generation of patient-specific iPS cells has the advantage of avoiding many of the ethical concerns associated with the use of embryonic or foetal material, and have no risk of immune rejection. Currently, several therapeutic relevant cell types, including motor neuron (Dimos et al., 2008), hepatocytes (Song et al., 2009), pancreatic insulin producing cells (Zhang et al., 2006), haematopoietic cells (Hanna et al., 2007), retinal cells (Carr et al., 2009), cardiomyocytes (Zwi et al., 2009) and MSCs (Lian et al., 2010), have been successfully derived from human iPS cells, and some of them have been tested to treat diseases in animal models. The use of iPS cells has thus been proposed as diagnostic and therapeutic tools for different haematological disorders (Ye et al., 2009). Nelson et al. (Nelson et al., 2009) reported the use of iPS cells for myocardial repair in animal models of acute myocardial infarction. There are several major challenges to overcome before iPS cell technology is applied in clinical practice. Now, current iPS cells are not "clinical grade". Genome-integrating viral vectors used for reprogramming are known oncogenes, particularly c-Myc, Oct4 and Klf4, such that iPS cells thus generated are unlikely to be safe for clinical application. Nonetheless recent technological advances, including reprogramming without viral integration such as plasmids or direct reprogramming protein delivery assays can solve this problem (Kiskinis and Eggan, 2010; Saha and Jaenisch, 2009). Despite the challenges in the therapeutic use of iPS cells, preclinical studies have provided the proof-of-concept that patient-specific iPS cells can provide an unlimited cell source to produce massive therapeutic cell types, such as cardiomyocytes and MSCs, and can be prepared in an "off the- shelf " format for cell transplantation.

Given the many potential risks of applying autologous iPS cell treatment to human subjects, iPS cell therapies may encounter strict regulatory restrictions. For instance, it took Geron Corporation more than 6 years to receive approval from the Food and Drug Administration (FDA) for its human ES cell-derived neuronal cell (GRNOPC1) therapies in terms of cell product safety and reliability. Recently, a second company has presented an investigational new drug for a phase I/II trial using human ES cell-derived retinal pigment epithelial (RPE) cells to treat patients with Stargardt's Macular Dystrophy (SMD), one of the most common causes of juvenile blindness. The sponsoring company, Advanced Cell Technology (ACT), has performed years of testing to show that differentiated RPE cells can improve the visual performance of rats without adverse effects (e.g., teratomas) in hundreds of treated animals. Another issue that may hinder the clinical translation of iPS cell therapies is the economic feasibility of producing individualized iPS cell therapeutic products. The viability of a business model for patient-specific iPS treatment is still unknown. It may well be the case that few if any pharmaceutical companies will be able to produce cost-effective individualized iPS cell products tailored for a single patient at a time. To be commercially feasible, these cells will need to be made in standardized, large-scale production, and the individual needs or profiles of patients will need to be easily assessed to allow matching and wide distribution (Sun et al., 2010). Several groups have also begun the generation of patient-specific human iPS cell lines. Park et al. generated a library of patient-derived iPS lines from numerous disorders including Huntington's disease, juvenile diabetes mellitus, Down syndrome, muscular dystrophy, and several others (Park et al., 2008). Of particular

interest are iPS cells derived from neurodegenerative diseases (Dimos et al., 2008; Ebert et al., 2009). These iPS lines can be differentiated *in vitro* into the affected neuronal cell type, generating for the first time a model for idiopathic neurodegenerative disorders which can be screened in culture for the onset, cell autonomy, and contribution of environmental factors to the phenotype. Ultimately, if human neurodegenerative phenotypes can be recapitulated in iPS cell-derived culture models, these cells could be screened using chemical libraries to identify molecules that can arrest or even reverse the progression of these disorders.

5. Similarities and differences between ES and iPS cells

The comparison of iPS and ES cells revealed that these cells are very similar. The differentiation capacity of iPS cells seems to resemble that of ES cells; iPS derived somatic cells are comparable to those derived from ES cells. Several studies describe the derivation of a variety of cell types from murine and human iPS cells, among them cardiomyocytes, smooth muscle cells, hepatic cells and neurons with similar differentiation behaviour of iPS and ES cells. The transcriptome of iPS and ES cells was analyzed by Gene chip analysis; the results showed that these cells are very similar but they are not identical. Chin and co-workers compared the expression pattern of different human ES and iPS cells; they analysed histone modifications and the expression of non-coding miRNAs in both type of cells and they constructed a fingerprint of iPS cells that distinguishes them from ES pluripotent cells (Pfannkuche et al., 2010). The comparison of the transcriptome of early and late passage iPS cells with ES cells revealed two datasets of differentially regulated genes. They identified a subset of 318 genes differentially expressed between human ES and iPS cells at any stage. The genes that are higher expressed in iPS cells were also found higher expressed in fibroblasts than in ES cells. The same conclusion is valid for genes that were expressed at a lower level in iPS than ES cells are usually also lower expressed in fibroblasts than ES cells. Together, these findings point at an imperfect reprogramming of a small set of genes. It is not known if there are implications of iPS fingerprint for the physiology of these cells. It is important determine the genes that are differentially regulated and that could influence the cellular physiology. It is likely that some of these gene functions are redundant with others that are not affected by incomplete reprogramming and, therefore, do not influence cell physiology (Pfannkuche et al., 2010).

Beside genes that constitute a potential iPS cell fingerprint, there are varying gene sets that are differentially expressed in individual iPS cell lines. It will be interesting to see if properties of the ancestral cell types are transmitted to the iPS cell line generated. In this regard, partial reprogramming plays a role and one fascinating aspect to address is if partial reprogramming alters the differentiation capacity of a cell in a way that it potentially influences the fate decision of the partially reprogrammed iPS cells. The differences between iPS and ES cells could be an assays to measure the quality of iPS cells. Although it has been shown that the overall gene expression of iPS cells differs from normal ES cells, this comparison has never been made between cells from the same individual. Usually iPS cells are compared with those ES cells either derived from another species or from a different individual; raising concerns about whether these are informative approaches. It is clear, therefore, that iPS cells derived from the trophoblast of an embryo compared with ES cells derived from the inner cell mass of the same embryo would give a more explicit view of how distinct or similar these cells really are (Pfannkuche et al., 2010).

6. Adult stem cells versus ESC&iPS

Although ESCs and iPS cells are now the most studied cells for clinical applications in neuromuscular diseases, different stem cells isolated from adult tissues was extensively used – and are still used – unfortunately with poor results. For several years after they were discovered, the satellite cell were considered as the only cells responsible for the growth and maintenance of skeletal muscle. With the improvements of cell-isolation technology, a number of markers were described to identify muscular and non-muscular subpopulations able to actively participate in myogenesis (Meregalli et al., 2010). In the skeletal muscle, adult multi-lineage progenitor cell populations were showed to have myogenic potential, such as muscle-derived stem cells (MDSCs) and muscle-derived CD133+ progenitors. Moreover, it was also shown that non-muscular resident stem cells could participate in myogenesis (Krause et al., 2001; Mezey et al., 2000; Pittenger et al., 1999; Prockop, 1997). In particular, a subpopulation of CD133+ cells was isolated form the blood, playing an important role in myogenic development (Torrente et al., 2003b) while mesoangioblasts were identified in the dorsal aorta of avian and mammalian species (Cossu and Bianco, 2003).

6.1 Satellite cells

Satellite cells are small progenitor cells that lie between the basement membrane and sarcolemma of individual muscle fibers: normally they are quiescent, consequently they cannot differentiate nor undergo cell division. Oxidative stress and specific stimuli form the environment can activate them, so that they differentiate, proliferate as skeletal myoblasts and activate myogenic differentiation to form new myofibers. Recently, Montarras and colleagues were able to directly isolate a pure population of satellite cells from diaphragm muscle of a Pax3-GFP knock-in mouse (Montarras et al., 2005). After FACS and gene expression analysis, they purified a predominantly quiescent population of satellite cells expressing Pax3, CD34 and Pax7. These cells were firstly injected into dystrophic dogs and restored dystrophin expression 3 weeks post-transplantation. Transplanted into irradiated dystrophic mice, they also formed a small amount of the satellite cell pool that expressed both Pax7 and Pax3 (Montarras et al., 2005). If compared with the results obtained after the injection of human cells isolated from adult muscle, these cells showed an incredibly efficient level of muscular regeneration (Morgan et al., 1996). Since they were doubtless highly myogenic, satellite cells were not considered in a clinical point of view, because it was difficult to isolate them and above all to proliferate and expand them to obtain the right number for transplantation experiments. Moreover, the growth of freshly isolated satellite cells in vitro significantly reduced their in vivo myogenic potential.

6.2 Muscle-derived stem cells (MDSCs)

Muscle-derived stem cells (MDSCs) are a recently-identified subpopulation of cells that resides within skeletal muscle and possess the ability to self renew and to differentiate into other mesodermal cell types (Sarig et al., 2006; Tamaki et al., 2007). Furthermore, it's known that these cells are distinct from satellite cells (Asakura and Rudnicki, 2002; Qu-Petersen et al., 2002) and that, when appropriately stimulated, they could preserve their myogenic potential in vitro even after differentiation into other lineages (Negroni et al., 2006). In the last years, different works assessed the capacity of MDSCs to differentiate and regenerate skeletal muscle when transplanted into animal models. Sca-1+CD34+ stem cells were purified from the muscle tissues of newborn mice, showing multipotency in vitro.

Moreover, after intra-arterial injection, these cells were able to interact and firmly adhere to endothelium in mdx muscles microcirculation and then participated in muscle regeneration (Torrente et al., 2003a). Qu-Petersen and collaborators isolated a MDSCs population Sca-1+/-CD34+/-c-kit-CD45- and demonstrated that they displayed a better transplantation efficiency than satellite cells (Qu-Petersen et al., 2002). MDSCs were also identified in human muscle, expressing in proliferation the CD133 antigen and also desmin and α-SMA when cultured in myogenic conditions (Miraglia et al., 1997). Moreover, among human MDSCs, it was identified a subpopulation of progenitor stem cells with neurogenic properties (Alessandri et al., 2004). According to these evidences, MDSCs are suitable for clinical perspectives as they are easy to proliferate, migrate through the vasculature, and are multipotent, although it could be necessary to better investigate their physiological location (Deasy et al., 2005; Deasy et al., 2001).

6.3 Mesoangioblasts

Mesoangioblasts are multipotent progenitors of mesodermal tissues, physically associated with the embryonic dorsal aorta in avian and mammalian species, expressing Flk-1, stem cell antigen 1, CD34 and various leukocyte molecules (Cossu and Bianco, 2003; Tagliafico et al., 2004). It was shown that mesoangioblasts treated with a lentiviral vector expressing human microdystrophin were able to produce dystrophin-positive myofibers after injection in animal model of DMD and ameliorated muscle function and mobility (Cossu and Sampaolesi, 2007; Sampaolesi et al., 2006). Furthermore, to improve their efficiency of muscle repair, mesoangioblasts were treated to increase their migration to skeletal muscle and to reduce unspecific trapping in the capillary filters of the body, such as liver and lung (Galvez et al., 2006).

6.4 CD133+ stem cells

A role for CD133 as a marker of stem cells with the capacity to engraft and differentiate to form functional non-haematopoietic adult lineages and contribute to disease amelioration via tissue regeneration emerged in the last years. Human CD133+ cells, isolated from peripheral blood and manipulated *in vitro* to undergo myogenesis, were shown to ameliorate disease via a direct contribution to muscular regeneration when transplanted into dystrophic mice (Torrente et al., 2004). In particular, they restored dystrophin expression and eventually regenerate the murine satellite cells pool after intramuscular and intra-arterial delivery. Human CD133+ cells colonized the mouse muscle and formed hybrid regenerated fibers expressing human dystrophin. Moreover, they were detected in several vessels near areas of regeneration, where they expressed human ve-cadherin and CD31 (Torrente et al., 2004). A CD133+ stem cell subpopulation was also identified in normal and dystrophic muscles. They were positive for CD45 antigen, indicating their hematopoietic commitment while the expression of Pax-7, Myf-5, MyoD, m-cadherin, MRF-4, and myogenin after 24 days of culture in the proliferation medium and their ability to differentiate into multinucleated myotubes expressing MyHCs suggested a myogenic commitment (Torrente et al., 2007). According to these data, CD133+ stem cells were considered as a possible tool in the treatment of degenerating diseases. Stamm and collaborators showed that transplanted BM-derived CD133+ cells improved function of infracted myocardium probably as a result of the amelioration in blood vessel formation (Stamm et al., 2003) while Torrente and co-workers demonstrated that intramuscular transplantation of muscle-derived CD133+ cells in DMD patients was a safe procedure

and feasible. DMD patients showed an increased number of capillaries per muscle fiber and expressed a change in the ratio of slow-to-fast myosin myofibers (Torrente et al., 2007). Human dystrophic blood- and muscle-derived CD133+ expressed an exon-skipped version of human dystrophin after transduction with a lentivirus carrying a construct designed to skip exon 51 and participated in vivo in muscle regeneration (Benchaouir et al., 2007). This combination of cell- and gene-based approaches via the *ex vivo* introduction of corrective genes into dystrophic CD133+ cells permitted – in a clinical point of view- the use of patient's own cells: autologous transplantation would reduce the risk of implant rejection.

7. Therapeutic potential of adult and embryonic stem cells

Several questions remain to be answered before any of the previously described cell preparations can be moved into clinical trials even if there has been great advance in the generation of cell populations showed *in vivo* myogenic potential. Based on their unique characteristics and in vivo skeletal muscle regeneration potential, adult stem cell populations discussed in this review are excellent clinical candidates. As shown before, mesoangioblasts (Cossu and Bianco, 2003) and blood derived CD133+ (Gavina et al., 2006) have the ability to migrate through the vasculature, most do not. Potential future methods to increase the migratory ability of stem cell population include the identification of cell surface markers like adhesion molecules (Torrente et al., 2003b) and appropriate growth factors (Horsley et al., 2003; Torrente et al., 2003b). Mesoangioblasts serve as a paradigm for widespread distribution, and after treatment with growth factors are able to correct efficiently the dystrophic phenotype. For now the intra-arterial injection of mesoangioblasts represent a hope for patients suffering from various muscular dystrophies. Satellite cells was one of the first cell types used in cell-based therapy of muscular dystrophy. Expanded satellite cells or myoblasts were isolated from wild-type mice and intramuscularly injected in dystrophic mdx mice (Conway et al., 1997; Tremblay et al., 1998); unfortunately it was demonstrated that myoblast transplantation is an inefficient technique because of the low efficiency of the dystrophin production in muscle fibers of DMD patients and no functional or clinical improvement in the children (Peault et al., 2007). In possible future clinical trials, adult stem cells purified from patients suffering for neuromuscular disorders could be ex vivo engineered and re-injected in the initial donor intra-arterially. The intra-arterial injections of the patient's own stem cells transduced allow the distribution of the cells to the whole body musculature so that it could be possible to take care of severe-affected patients that have reduced mass body, as in DMD and BDM pathology (Brignier and Gewirtz, 2010). One of the most important problem to solve for future clinical application is the amelioration in safety procedures of gene's modifications. One of the most reliable methods for gene therapy, fully utilized in DMD clinical approaches, seems to be the exon skipping mediated by AONs or molecules like PTC124. Ongoing phase I/II studies try to assess the efficacy and the safety of intramuscular administered morpholino oligomer directed against exon 51 (AVI-4658 PMO). Morpholinos can interfere with mRNA splicing processes by preventing the formation of the snRNP complex or by interfering with the binding sites for other regulatory proteins (Vetrini et al., 2006). They mediate the exclusion of exons from the mature mRNA as AONs. PTC124 was shown to partially restore dystrophin production in animals with DMD due to a nonsense mutation. The main purpose of a phase II study completed on May 2007 was to understand whether PTC124 can safely increase functional

dystrophin protein in the muscles of patients with DMD due to a nonsense mutations. This study demonstrated the safety and the efficacy of the PTC124 treatment; now three ongoing phase 2a and 2b studies are started in DMD and BMD patients (www.clinicaltrials.gov).
A decade of studies in human ESCs has yielded remarkable progress and understanding in stem cell biology. The technical challenge of creating patient-specific ESCs, the ethical issues arising from the foetal origin of human ESCs and the potential risk of immune rejection make broad clinical application of this cell type difficult. Recent advances in human iPS cell technology can potentially circumvent these disadvantages: iPS cells thus provide an invaluable resource of cell types for modelling diseases, drug or toxicology screening, and patient-specific cell therapy. Significant challenges remain to be overcome before the full potential of human iPS cell technology can be realised. The utilization and practical application of ESC in cell replacement therapy are still in a preliminary stage and need more investigation and clinical trials before they can be accepted as ideal for the treatment of neuromuscular diseases. Nevertheless, the daily increase in experimental findings is reinforcing the hope that ESC will be a versatile source of renewable cells for application in cell replacement therapy (Brignier & Gewirtz, 2010). Therefore, there is enough optimism among the scientists that ESC-based therapies may offer reliable and cost-effective therapeutic substitute for treatment of severe degenerative disorders in the near future. Major objection to hES cell research is focused one ethical reasons. The core reason for objection to hES cell research is that it destroys human blastocysts or embryos, which means it destroys human lives and eventually violates human dignity because of the blastosysts have the same moral value as that of human beings or at least that blastocysts have the potential to develop into human beings (Jung, 2009). Accordingly, research with hESCs is increasing exponentially worldwide, particularly in the United States, where important limitations on research with such cells were overturned in 2009. Furthermore, the US Food and Drug Administration trial using hESC-based therapy in patients with spinal cord injury is now on-going. Nonetheless, a number of substantive scientific and ethical issues remain to be resolved before hESCs can enter the therapeutic mainstream. In the meantime, recent breakthroughs in generating iPSCs would obviate the need to solve the most vexing of these problems. In fact, it seems reasonable to hope that in the next few years many of the enabling issues relevant to iPSCs will be solved, allowing the field of regenerative medicine to deliver on its vast potential promise. Although it is difficult to predict the ultimate utility of stem cell–based therapy at this time, it is not difficult to conclude that this is an extremely important area of scientific research. Open discussions between political bodies and the various interest groups in the scientific, medical, and religious communities need to take place to address the concerns of each and to provide an ultimate solution that is clearly in the interest of humanity.

8. Conclusions

European Medicines Agency (EMEA) issued the guideline to replace the Points to Consider on the Manufacture and Quality Control of Human Somatic Cell Therapy Medicinal Products (CPMP/BWP/41450/98). In general, when a cell-based medicinal product (CBMP) enters the clinical development phase, the same requirements as for other medicinal products apply. The clinical development plan should include pharmacodynamic studies, pharmacokinetic studies, mechanism of action studies, dose finding studies and randomised clinical trials in accordance to the Directive 2001/20/EC and to the existing general

guidances and specific guidances for the condition evaluated. It takes into account the current legislation and the heterogeneity of human cell-based products, including combination products. A risk analysis approach can be used by the applicants to justify the development and evaluation plans and can be a basis for the preparation of a risk management plan. Special problems might be associated with the clinical development of human cell-based medicinal products. Guidance is therefore provided on the conduct of pharmacodynamic/pharmacokinetic studies, dose finding and clinical efficacy and safety studies. The guideline describes the special consideration that should be given to pharmacovigilance aspects and the risk management plan for these products. The active substance of a CBMP is composed of the engineered (manipulated) cells and/or tissues. When the cells in the active substance are genetically modified, the "Note for Guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products" should be followed, which gives details on the quality control, characterisation and preclinical testing of gene transfer vectors. Cell populations which are transformed should be assayed for appropriate and reproducible expression of the newly acquired characteristics. Special attention should be paid to the level and length of expression and quality of the gene product(s) produced by the cells. As far as applicable and practicable, the new characteristics of the cells should be quantified and controlled. During in vitro cell culture, consideration should be given to ensure acceptable growth and manipulation of the isolated cells. The processing steps should be properly designed to preserve the integrity and control the function of the cells and their manipulation should be documented in detail and closely monitored according to specific process controls. Moreover, the duration of cell culture and maximum number of cell passages should be clearly specified and validated. The relevant genotypic and phenotypic characteristics of the primary cell cultures, of the established cell lines and the derived cell clones should be defined and their stability with respect to culture longevity determined. Consistency/repeatability of the cell culture process should be demonstrated and the culture conditions including the media and the duration should be optimised with respect to the intended clinical function of the cells. If genetically modified cells are used in the product, any additional proteins expressed from the vector, such as antibiotic resistance factors, selection markers, should be analysed to determine their presence in the product. Microassay for gene expression profile, flow cytometry and other different techniques allowed these expression studies. CBMP might require administration through specific surgical procedures, method of administration or the presence of concomitant treatments to obtain the intended therapeutic effect. The biological effects of CBMP are highly dependent on the *in vivo* environment, and may be influenced by the replacement process or the immune reaction either from the patient or from the cell-based product. These requirements coming from the clinical development should be taken into account for the final use of these products. Their standardisation and optimisation should be an integral part of the clinical development studies. Ahead of these considerations it's not still provided a reproducible method to isolate ESCs even if an ES cell–based therapy would have many advantages: it could allow the transplantation of a more primitive cell with greater replicative potential and patient-specific ES cells could be induced from adult somatic cells. The development of several ESC-based technologies, such as genetic manipulation tools and their potential applications, could accelerate the use of these cells into clinical therapy, even if ethical, logistics and economics concerns need attention in case of ESC-based techniques. There are several major challenges to overcome before iPS cell technology is applied in clinical practice. First, current iPS cells are not "clinical grade".

Genome-integrating viral vectors used for reprogramming are known oncogenes, particularly c-Myc, Oct4 and Klf4, such that iPS cells thus generated are unlikely to be safe for clinical application. Nonetheless recent technological advances, including reprogramming without viral integration such as plasmids or direct reprogramming protein delivery assays could solve this problem (Kiskinis and Eggan, 2010; Saha and Jaenisch, 2009). Despite the challenges in the therapeutic use of iPS cells, preclinical studies provided the proof-of-concept that patient-specific iPS cells can provide an unlimited cell source to produce massive therapeutic cell types, such as cardiomyocytes and MSCs, and can be prepared in an "off the- shelf " format for cell transplantation. However given the many potential risks of applying autologous iPS cell treatment to human subjects, iPS cell therapies may encounter strict regulatory restrictions.

At now in our opinion the most promising results in the treatment of neuromuscular disorders were obtained using adult stem cells because of many questions are needed to be answer regarding the ES and iPS cells. According to the results described, the most promising possibility for the therapy of muscular dystrophies is a combination of different approaches to obtain the beneficial impact of multiple strategies combined into a single approach, such as cellular therapy associated with gene therapy or pharmacological treatments. One of the most used approach is called autologous transfer in which patient's own cells are genetically corrected in vitro with lentiviral vectors and then re-implanted to allow the re-expression of functional dystrophin protein. The 'exon skipping' approach is an alternative strategy for gene therapy and it is done through AONs that hybridize with the donor and/or acceptor sites of the mutated exon, causing its exclusion from the intact transcript. On the other side, the allogenic transfer implies that the cells isolated from an individual with functional dystrophin will be injected into the patient, allowing problems due to immunorejections or administration of specific immunosuppressive drugs. Several problems arose quickly, such as the low efficiency with which stem cells enter muscle via vasculature, the potential to enhance proliferation of stem cells in culture, the time required in culture for autologous cells prior to implantation back into the patient, the longevity of the transplanted muscle nuclei in vivo, the development of tumours as a consequence of hazardous integration of the provirus. In conclusion the success of the clinical application of adult or embryonic stem cells will be employed to a large-scale production of desired cell type with appropriate functionality, an optimal number of cells for transplant, a modification of less invasive delivery systems and a techniques to label cells for transplant and subsequent tracking of cell fate.

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10. References

Alessandri, G.; S. Pagano, A. Bez, A. Benetti, S. Pozzi, G. Iannolo, M. Baronio, G. Invernici, A. Caruso, C. Muneretto, G. Bisleri, & Parati, E. (2004). Isolation and culture of

human muscle-derived stem cells able to differentiate into myogenic and neurogenic cell lineages. *Lancet*. 364:1872-83.

Amit, M. & Itskovitz-Eldor, J. (2002). Derivation and spontaneous differentiation of human embryonic stem cells. *J Anat*. 200:225-32.

Andrews, P.W.; J. Casper, I. Damjanov, M. Duggan-Keen, A. Giwercman, J. Hata, A. von Keitz, L.H. Looijenga, J.L. Millan, J.W. Oosterhuis, M. Pera, M. Sawada, H.J. Schmoll, N.E. Skakkebaek, W. van Putten, & Stern P. (1996). Comparative analysis of cell surface antigens expressed by cell lines derived from human germ cell tumours. 1*Int J Cancer*. 66:806-16.

Asakura, A. & M.A. Rudnicki M.A. (2002). Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation. *Exp Hematol*. 30:1339-45.

Benchaouir, R.; M. Meregalli, A. Farini, G. D'Antona, M. Belicchi, A. Goyenvalle, M. Battistelli, N. Bresolin, R. Bottinelli, L. Garcia, & Torrente Y. (2007). Restoration of human dystrophin following transplantation of exon-skipping-engineered DMD patient stem cells into dystrophic mice. *Cell Stem Cell*. 1:646-57.

Berrih, S.; E. Morel, C. Gaud, F. Raimond, H. Le Brigand, & Bach J.F. (1984). Anti-AChR antibodies, thymic histology, and T cell subsets in myasthenia gravis. *Neurology*. 34:66-71.

Bertoni, C. (2008). Clinical approaches in the treatment of Duchenne muscular dystrophy (DMD) using oligonucleotides. *Front Biosci*. 13:517-27.

Bhagavati, S. & Xu W. (2005). Generation of skeletal muscle from transplanted embryonic stem cells in dystrophic mice. *Biochem Biophys Res Commun*. 333:644-9.

Biggar, W.D.; V.A. Harris, L. Eliasoph, & Alman B. (2006). Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. *Neuromuscul Disord*. 16:249-55.

Brignier, A.C. & Gewirtz A.M. (2010). Embryonic and adult stem cell therapy. *J Allergy Clin Immunol*. 125:S336-44.

Byrne, J.A.; D.A. Pedersen, L.L. Clepper, M. Nelson, W.G. Sanger, S. Gokhale, D.P. Wolf, and Mitalipov SM. (2007). Producing primate embryonic stem cells by somatic cell nuclear transfer. *Nature*. 450:497-502.

Campbell, K.H.; J. McWhir, W.A. Ritchie, & Wilmut I. (1996). Sheep cloned by nuclear transfer from a cultured cell line. *Nature*. 380:64-6.

Carpenter, M.K.; E. Rosler, & Rao M.S. (2003). Characterization and differentiation of human embryonic stem cells. *Cloning Stem Cells*. 5:79-88.

Carr, A.J.; A.A. Vugler, S.T. Hikita, J.M. Lawrence, C. Gias, L.L. Chen, D.E. Buchholz, A. Ahmado, M. Semo, M.J. Smart, S. Hasan, L. da Cruz, L.V. Johnson, D.O. Clegg, & Coffey P.J. (2009). Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. *PLoS One*. 4:e8152.

Caspi, O.; A. Lesman, Y. Basevitch, A. Gepstein, G. Arbel, I.H. Habib, L. Gepstein, & Levenberg S. (2007). Tissue engineering of vascularized cardiac muscle from human embryonic stem cells. *Circ Res*. 100:263-72.

Charwat, S.; I. Lang, M. Dettke, S. Graf, N. Nyolczas, R. Hemetsberger, S. Zamini, A. Khorsand, H. Sochor, G. Maurer, D. Glogar, & Gyongyosi M. (2010). Effect of intramyocardial delivery of autologous bone marrow mononuclear stem cells on the regional myocardial perfusion. NOGA-guided subanalysis of the MYSTAR prospective randomised study. *Thromb Haemost*. 103:564-71.

Chuang, C.K.; L.Y. Sung, S.M. Hwang, W.H. Lo, H.C. Chen, & Hu Y.C. (2007). Baculovirus as a new gene delivery vector for stem cell engineering and bone tissue engineering. *Gene Ther*. 14:1417-24.

Conrad, S.; M. Renninger, J. Hennenlotter, T. Wiesner, L. Just, M. Bonin, W. Aicher, H.J. Buhring, U. Mattheus, A. Mack, H.J. Wagner, S. Minger, M. Matzkies, M. Reppel, J. Hescheler, K.D. Sievert, A. Stenzl, & Skutella T. (2008). Generation of pluripotent stem cells from adult human testis. *Nature*. 456:344-9.

Conway, S.J.; D.J. Henderson, & Copp A.J. (1997). Pax3 is required for cardiac neural crest migration in the mouse: evidence from the splotch (Sp2H) mutant. *Development*. 124:505-14.

Cossu, G. & Bianco P. (2003). Mesoangioblasts--vascular progenitors for extravascular mesodermal tissues. *Curr Opin Genet Dev*. 13:537-42.

Cossu, G. & Sampaolesi M. (2007). New therapies for Duchenne muscular dystrophy: challenges, prospects and clinical trials. *Trends Mol Med*. 13:520-6.

Darabi, R.; K. Gehlbach, R.M. Bachoo, S. Kamath, M. Osawa, K.E. Kamm, M. Kyba, & Perlingeiro R.C. (2008a). Functional skeletal muscle regeneration from differentiating embryonic stem cells. *Nat Med*. 14:134-43.

Darabi, R.; F.N. Santos, & Perlingeiro R.C. (2008b). The therapeutic potential of embryonic and adult stem cells for skeletal muscle regeneration. *Stem Cell Rev*. 4:217-25.

Deasy, B.M.; B.M. Gharaibeh, J.B. Pollett, M.M. Jones, M.A. Lucas, Y. Kanda, & Huard J. (2005). Long-term self-renewal of postnatal muscle-derived stem cells. *Mol Biol Cell*. 16:3323-33.

Deasy, B.M.; R.J. Jankowski, & Huard J. (2001). Muscle-derived stem cells: characterization and potential for cell-mediated therapy. *Blood Cells Mol Dis*. 27:924-33.

Dimos, J.T.; K.T. Rodolfa, K.K. Niakan, L.M. Weisenthal, H. Mitsumoto, W. Chung, G.F. Croft, G. Saphier, R. Leibel, R. Goland, H. Wichterle, C.E. Henderson, & Eggan K. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 321:1218-21.

Drachman, D.B. (1994). Myasthenia gravis. *N Engl J Med*. 330:1797-810.

Ebert, A.D.; J. Yu, F.F. Rose, Jr., V.B. Mattis, C.L. Lorson, J.A. Thomson, & Svendsen C.N. (2009). Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*. 457:277-80.

Endo, T. (2007). Stem cells and plasticity of skeletal muscle cell differentiation: potential application to cell therapy for degenerative muscular diseases. *Regen Med*. 2:243-56.

Esteban, M.A.; T. Wang, B. Qin, J. Yang, D. Qin, J. Cai, W. Li, Z. Weng, J. Chen, S. Ni, K. Chen, Y. Li, X. Liu, J. Xu, S. Zhang, F. Li, W. He, K. Labuda, Y. Song, A. Peterbauer, S. Wolbank, H. Redl, M. Zhong, D. Cai, L. Zeng, & Pei J. (2010). Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. *Cell Stem Cell*. 6:71-9.

Feng, B.; J. Jiang, P. Kraus, J.H. Ng, J.C. Heng, Y.S. Chan, L.P. Yaw, W. Zhang, Y.H. Loh, J. Han, V.B. Vega, V. Cacheux-Rataboul, B. Lim, T. Lufkin, & Ng H.H. (2009). Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. *Nat Cell Biol*. 11:197-203.

Ferreira, L.S.; S. Gerecht, J. Fuller, H.F. Shieh, G. Vunjak-Novakovic, & Langer R. (2007). Bioactive hydrogel scaffolds for controllable vascular differentiation of human embryonic stem cells. *Biomaterials*. 28:2706-17.

Fuchs, E. & Segre J.A. (2000). Stem cells: a new lease on life. *Cell*. 100:143-55.

Galvez, B.G.; M. Sampaolesi, S. Brunelli, D. Covarello, M. Gavina, B. Rossi, G. Constantin, Y. Torrente, & Cossu G. (2006). Complete repair of dystrophic skeletal muscle by mesoangioblasts with enhanced migration ability. *J Cell Biol*. 174:231-43.

Gaspar-Maia, A.; A. Alajem, F. Polesso, R. Sridharan, M.J. Mason, A. Heidersbach, J. Ramalho-Santos, M.T. McManus, K. Plath, E. Meshorer, & Ramalho-Santos M.

(2009). Chd1 regulates open chromatin and pluripotency of embryonic stem cells. *Nature*. 460:863-8.

Gavina, M.; M. Belicchi, B. Rossi, L. Ottoboni, F. Colombo, M. Meregalli, M. Battistelli, L. Forzenigo, P. Biondetti, F. Pisati, D. Parolini, A. Farini, A.C. Issekutz, N. Bresolin, F. Rustichelli, G. Constantin, & Torrente Y. (2006). VCAM-1 expression on dystrophic muscle vessels has a critical role in the recruitment of human blood-derived CD133+ stem cells after intra-arterial transplantation. *Blood*. 108:2857-66.

Hamed, S.A. (2006). Drug evaluation: PTC-124--a potential treatment of cystic fibrosis and Duchenne muscular dystrophy. *IDrugs*. 9:783-9.

Hanna, J.; M. Wernig, S. Markoulaki, C.W. Sun, A. Meissner, J.P. Cassady, C. Beard, T. Brambrink, L.C. Wu, T.M. Townes, & Jaenisch R. (2007). Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science*. 318:1920-3.

Hay, D.C.; L. Sutherland, J. Clark, & Burdon T. (2004). Oct-4 knockdown induces similar patterns of endoderm and trophoblast differentiation markers in human and mouse embryonic stem cells. *Stem Cells*. 22:225-35.

Helg, C.; B. Chapuis, J.F. Bolle, P. Morel, D. Salomon, E. Roux, V. Antonioli, M. Jeannet, & Leski M. (1994). Renal transplantation without immunosuppression in a host with tolerance induced by allogeneic bone marrow transplantation. *Transplantation*. 58:1420-2.

Hoch, W.; J. McConville, S. Helms, J. Newsom-Davis, A. Melms, & Vincent A. (2001). Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med*. 7:365-8.

Hochedlinger, K.; & Jaenisch R. (2003). Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. *N Engl J Med*. 349:275-86.

Hoffman, L.M. & Carpenter M.K. (2005). Characterization and culture of human embryonic stem cells. *Nat Biotechnol*. 23:699-708.

Horsley, V.; K.M. Jansen, S.T. Mills, & Pavlath G.K. (2003). IL-4 acts as a myoblast recruitment factor during mammalian muscle growth. *Cell*. 113:483-94.

Hotta, A. & Ellis J. (2008). Retroviral vector silencing during iPS cell induction: an epigenetic beacon that signals distinct pluripotent states. *J Cell Biochem*. 105:940-8.

Huangfu, D.; K. Osafune, R. Maehr, W. Guo, A. Eijkelenboom, S. Chen, W. Muhlestein, & Melton D.A. (2008). Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol*. 26:1269-75.

Hyser, C.L. & Mendell J.R. (1988). Recent advances in Duchenne and Becker muscular dystrophy. *Neurol Clin*. 6:429-53.

Hyslop, L.A.; L. Armstrong, M. Stojkovic, & Lako M. (2005). Human embryonic stem cells: biology and clinical implications. *Expert Rev Mol Med*. 7:1-21.

Jaenisch, R. & Young R. (2008). Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*. 132:567-82.

Jiang, Y.; B.N. Jahagirdar, R.L. Reinhardt, R.E. Schwartz, C.D. Keene, X.R. Ortiz-Gonzalez, M. Reyes, T. Lenvik, T. Lund, M. Blackstad, J. Du, S. Aldrich, A. Lisberg, W.C. Low, D.A. Largaespada, & Verfaillie C.M. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 418:41-9.

Joannides, A.J.; C. Fiore-Heriche, A.A. Battersby, P. Athauda-Arachchi, I.A. Bouhon, L. Williams, K. Westmore, P.J. Kemp, A. Compston, N.D. Allen, & Chandran S. (2007). A scaleable and defined system for generating neural stem cells from human embryonic stem cells. *Stem Cells*. 25:731-7.

Jung, K.W. (2009). Perspectives on human stem cell research. *J Cell Physiol*. 220:535-7.

Kaji, K.; K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni, & Woltjen K. (2009). Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature*. 458:771-5.

Kamochi, H.; M.S. Kurokawa, H. Yoshikawa, Y. Ueda, C. Masuda, E. Takada, K. Watanabe, M. Sakakibara, Y. Natuki, K. Kimura, M. Beppu, H. Aoki, & Suzuki N. (2006). Transplantation of myocyte precursors derived from embryonic stem cells transfected with IGFII gene in a mouse model of muscle injury. *Transplantation*. 82:516-26.

Kannagi, R.; N.A. Cochran, F. Ishigami, S. Hakomori, P.W. Andrews, B.B. Knowles, & Solter D. (1983a). Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. *Embo J*. 2:2355-61.

Kannagi, R.; S.B. Levery, F. Ishigami, S. Hakomori, L.H. Shevinsky, B.B. Knowles, & Solter D. (1983b). New globoseries glycosphingolipids in human teratocarcinoma reactive with the monoclonal antibody directed to a developmentally regulated antigen, stage-specific embryonic antigen 3. *J Biol Chem*. 258:8934-42.

Kim, D.; C.H. Kim, J.I. Moon, Y.G. Chung, M.Y. Chang, B.S. Han, S. Ko, E. Yang, K.Y. Cha, R. Lanza, & Kim K.S. (2009a). Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell*. 4:472-6.

Kim, J.B.; V. Sebastiano, G. Wu, M.J. Arauzo-Bravo, P. Sasse, L. Gentile, K. Ko, D. Ruau, M. Ehrich, D. van den Boom, J. Meyer, K. Hubner, C. Bernemann, C. Ortmeier, M. Zenke, B.K. Fleischmann, H. Zaehres, & Scholer H.R. (2009b). Oct4-induced pluripotency in adult neural stem cells. *Cell*. 136:411-9.

Kiskinis, E. & Eggan K. (2010). Progress toward the clinical application of patient-specific pluripotent stem cells. *J Clin Invest*. 120:51-9.

Klimanskaya, I.; Y. Chung, S. Becker, S.J. Lu, & Lanza R. (2006). Human embryonic stem cell lines derived from single blastomeres. *Nature*. 444:481-5.

Kopp, J.L.; B.D. Ormsbee, M. Desler, & Rizzino A. (2008). Small increases in the level of Sox2 trigger the differentiation of mouse embryonic stem cells. *Stem Cells*. 26:903-11.

Krause, D.S.; N.D. Theise, M.I. Collector, O. Henegariu, S. Hwang, R. Gardner, S. Neutzel, Sharkis S.J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*. 105:369-77.

Lafreniere, J.F.; P. Mills, M. Bouchentouf, & Tremblay J.P. (2006). Interleukin-4 improves the migration of human myogenic precursor cells in vitro and in vivo. *Exp Cell Res*. 312:1127-41.

Leist, M.; S. Bremer, P. Brundin, J. Hescheler, A. Kirkeby, K.H. Krause, P. Poerzgen, M. Puceat, M. Schmidt, A. Schrattenholz, N.B. Zak, & Hentze H. (2008). The biological and ethical basis of the use of human embryonic stem cells for in vitro test systems or cell therapy. *Altex*. 25:163-90.

Lian, Q.; Y. Zhang, J. Zhang, H.K. Zhang, X. Wu, Y. Zhang, F.F. Lam, S. Kang, J.C. Xia, W.H. Lai, K.W. Au, Y.Y. Chow, C.W. Siu, C.N. Lee, & Tse H.F. (2010). Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. *Circulation*. 121:1113-23.

Liao, J.; Z. Wu, Y. Wang, L. Cheng, C. Cui, Y. Gao, T. Chen, L. Rao, S. Chen, N. Jia, H. Dai, S. Xin, J. Kang, G. Pei, & Xiao L. (2008). Enhanced efficiency of generating induced pluripotent stem (iPS) cells from human somatic cells by a combination of six transcription factors. *Cell Res*. 18:600-3.

Lin, T.; R. Ambasudhan, X. Yuan, W. Li, S. Hilcove, R. Abujarour, X. Lin, H.S. Hahm, E. Hao, A. Hayek, & Ding S. (2009). A chemical platform for improved induction of human iPSCs. *Nat Methods*. 6:805-8.

Lin, T.; C. Chao, S. Saito, S.J. Mazur, M.E. Murphy, E. Appella, & Xu Y. (2005). p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat Cell Biol*. 7:165-71.

Lyssiotis, C.A.; R.K. Foreman, J. Staerk, M. Garcia, D. Mathur, S. Markoulaki, J. Hanna, L.L. Lairson, B.D. Charette, L.C. Bouchez, M. Bollong, C. Kunick, A. Brinker, C.Y. Cho, P.G. Schultz, & Jaenisch R. (2009). Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. *Proc Natl Acad Sci U S A*. 106:8912-7.

Maherali, N.; R. Sridharan, W. Xie, J. Utikal, S. Eminli, K. Arnold, M. Stadtfeld, R. Yachechko, J. Tchieu, R. Jaenisch, K. Plath, & Hochedlinger K. (2007). Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*. 1:55-70.

Marchesi, C & Pareyson, D. (2009). Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. *Lancet Neurol*. 8(7):654-67

McClorey, G.; A.M. Fall, H.M. Moulton, P.L. Iversen, J.E. Rasko, M. Ryan, S. Fletcher, & Wilton S.D. (2006). Induced dystrophin exon skipping in human muscle explants. *Neuromuscul Disord*. 16:583-90.

Melton, C.; R.L. Judson, & Blelloch R. (2010). Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature*. 463:621-6.

Meregalli, M.; A. Farini, D. Parolini, S. Maciotta, & Torrente Y. (2010). Stem cell therapies to treat muscular dystrophy: progress to date. *BioDrugs*. 24:237-47.

Meriggioli, M.N.; & Sanders D.B. (2009). Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol*. 8:475-90.

Mezey, E.; K.J. Chandross, G. Harta, R.A. Maki, & McKercher S.R. (2000). Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science*. 290:1779-82.

Miraglia, S.; W. Godfrey, A.H. Yin, K. Atkins, R. Warnke, J.T. Holden, R.A. Bray, E.K. Waller, & Buck D.W. (1997). A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood*. 90:5013-21.

Mitalipova, M.M.; R.R. Rao, D.M. Hoyer, J.A. Johnson, L.F. Meisner, K.L. Jones, S. Dalton, & Stice S.L. (2005). Preserving the genetic integrity of human embryonic stem cells. *Nat Biotechnol*. 23:19-20.

Montarras, D.; J. Morgan, C. Collins, F. Relaix, S. Zaffran, A. Cumano, T. Partridge, & Buckingham M. (2005). Direct isolation of satellite cells for skeletal muscle regeneration. *Science*. 309:2064-7.

Moon, S.Y.; Y.B. Park, D.S. Kim, S.K. Oh, & Kim D.W. (2006). Generation, culture, and differentiation of human embryonic stem cells for therapeutic applications. *Mol Ther*. 13:5-14.

Morgan, J.E.; R.M. Fletcher, & Partridge T.A. (1996). Yields of muscle from myogenic cells implanted into young and old mdx hosts. *Muscle Nerve*. 19:132-9.

Mountford, J.C. (2008). Human embryonic stem cells: origins, characteristics and potential for regenerative therapy. *Transfus Med*. 18:1-12.

Murry, C.E. & Keller G. (2008). Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell*. 132:661-80.

Nakagawa, M.; M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa, & Yamanaka S. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*. 26:101-6.

Negroni, E.; G.S. Butler-Browne, & Mouly V. (2006). Myogenic stem cells: regeneration and cell therapy in human skeletal muscle. *Pathol Biol (Paris)*. 54:100-8.

Nelson, T.J.; A. Martinez-Fernandez, S. Yamada, C. Perez-Terzic, Y. Ikeda, & Terzic A. (2009). Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation*. 120:408-16.

Niwa, H. (2001). Molecular mechanism to maintain stem cell renewal of ES cells. *Cell Struct Funct*. 26:137-48.

Niwa, H.; S. Masui, I. Chambers, A.G. Smith, & Miyazaki J. (2002). Phenotypic complementation establishes requirements for specific POU domain and generic transactivation function of Oct-3/4 in embryonic stem cells. *Mol Cell Biol*. 22:1526-36.

Niwa, H.; J. Miyazaki, & Smith A.G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat Genet*. 24:372-6.

Nowak, K.J. & Davies K.E. (2004). Duchenne muscular dystrophy and dystrophin: pathogenesis and opportunities for treatment. *EMBO Rep*. 5:872-6.

Okita, K.; T. Ichisaka, & Yamanaka S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature*. 448:313-7.

Owonikoko, T; M. Agha, R. Balassanian, R. Smith, & Raptis A. (2007). Gemtuzumab therapy for isolated extramedullary AML relapse following allogeneic stem-cell transplant. *Nat Clin Pract Oncol*. 4:491-5.

Pan, C.; B. Lu, H. Chen, & Bishop C.E. (2010). Reprogramming human fibroblasts using HIV-1 TAT recombinant proteins OCT4, SOX2, KLF4 and c-MYC. *Mol Biol Rep*. 37:2117-24.

Park, I.H.; R. Zhao, J.A. West, A. Yabuuchi, H. Huo, T.A. Ince, P.H. Lerou, M.W. Lensch, & Daley G.Q. (2008). Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 451:141-6.

Patrick, J. & Lindstrom J. (1973). Autoimmune response to acetylcholine receptor. *Science*. 180:871-2.

Peault, B.; M. Rudnicki, Y. Torrente, G. Cossu, J.P. Tremblay, T. Partridge, E. Gussoni, L.M. Kunkel, & Huard J. (2007). Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. *Mol Ther*. 15:867-77.

Pesce, M.; & Scholer H.R. (2001). Oct-4: gatekeeper in the beginnings of mammalian development. *Stem Cells*. 19:271-8.

Pfannkuche, K.; A. Fatima, M.K. Gupta, R. Dieterich, & Hescheler J. (2010). Initial colony morphology-based selection for iPS cells derived from adult fibroblasts is substantially improved by temporary UTF1-based selection. *PLoS One*. 5:e9580.

Pittenger, M.F.; A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, & Marshak D.R. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*. 284:143-7.

Price, F.D.; K. Kuroda, & Rudnicki M.A. (2007). Stem cell based therapies to treat muscular dystrophy. *Biochim Biophys Acta*. 1772:272-83.

Prockop, D.J. (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*. 276:71-4.

Qu-Petersen, Z.; B. Deasy, R. Jankowski, M. Ikezawa, J. Cummins, R. Pruchnic, J. Mytinger, B. Cao, C. Gates, A. Wernig, & Huard J. (2002). Identification of a novel population

of muscle stem cells in mice: potential for muscle regeneration. *J Cell Biol*. 157:851-64.

Reubinoff, B.E.; P. Itsykson, T. Turetsky, M.F. Pera, E. Reinhartz, A. Itzik, & Ben-Hur T. (2001). Neural progenitors from human embryonic stem cells. *Nat Biotechnol*. 19:1134-40.

Rideau, Y. ; B. Glorion, & Duport G. (1983). Prolongation of ambulation in the muscular dystrophies. *Acta Neurol (Napoli)*. 5:390-7.

Rideout, W.M. 3rd; K. Eggan, & Jaenisch R. (2001). Nuclear cloning and epigenetic reprogramming of the genome. *Science*. 293:1093-8.

Rideout, W.M. 3rd; K. Hochedlinger, M. Kyba, G.Q. Daley, & Jaenisch R. (2002). Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell*. 109:17-27.

Rizzino, A. (2009). Sox2 and Oct-3/4: a versatile pair of master regulators that orchestrate the self-renewal and pluripotency of embryonic stem cells. *Wiley Interdiscip Rev Syst Biol Med*. 1:228-36.

Rosner, M.H.; M.A. Vigano, K. Ozato, P.M. Timmons, F. Poirier, P.W. Rigby, & Staudt L.M. (1990). A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. *Nature*. 345:686-92.

Rowland, B.D. & Peeper D.S. (2006). KLF4, p21 and context-dependent opposing forces in cancer. *Nat Rev Cancer*. 6:11-23.

Roxanis, I.; K. Micklem, & Willcox N. (2001). True epithelial hyperplasia in the thymus of early-onset myasthenia gravis patients: implications for immunopathogenesis. *J Neuroimmunol*. 112:163-73.

Saha, K. & Jaenisch R. (2009). Technical challenges in using human induced pluripotent stem cells to model disease. *Cell Stem Cell*. 5:584-95.

Sampaolesi, M.; S. Blot, G. D'Antona, N. Granger, R. Tonlorenzi, A. Innocenzi, P. Mognol, J.L. Thibaud, B.G. Galvez, I. Barthelemy, L. Perani, S. Mantero, M. Guttinger, O. Pansarasa, C. Rinaldi, M.G. Cusella De Angelis, Y. Torrente, C. Bordignon, R. Bottinelli, & Cossu G. (2006). Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature*. 444:574-9.

Sarig, R.; Z. Baruchi, O. Fuchs, U. Nudel, & Yaffe D. (2006). Regeneration and transdifferentiation potential of muscle-derived stem cells propagated as myospheres. *Stem Cells*. 24:1769-78.

Shavlakadze, T.; J. White, J.F. Hoh, N. Rosenthal, & Grounds M.D. (2004). Targeted expression of insulin-like growth factor-I reduces early myofiber necrosis in dystrophic mdx mice. *Mol Ther*. 10:829-43.

Shi, Y.; Y. Zhao, & Deng H. (2010). Powering reprogramming with vitamin C. *Cell Stem Cell*. 6:1-2.

Singh, N.; V. Pillay, & Choonara Y.E. (2007). Advances in the treatment of Parkinson's disease. *Prog Neurobiol*. 81:29-44.

Siu, C.W.; S.Y. Liao, Y. Liu, Q. Lian, & Tse H.F. (2010). Stem cells for myocardial repair. *Thromb Haemost*. 104:6-12.

Skottman, H.; M.S. Dilber, & Hovatta O. (2006). The derivation of clinical-grade human embryonic stem cell lines. *FEBS Lett*. 580:2875-8.

Solter, D. (2000). Mammalian cloning: advances and limitations. *Nat Rev Genet*. 1:199-207.

Song, Z.; J. Cai, Y. Liu, D. Zhao, J. Yong, S. Duo, X. Song, Y. Guo, Y. Zhao, H. Qin, X. Yin, C. Wu, J. Che, S. Lu, M. Ding, & Deng H. (2009). Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. *Cell Res*. 19:1233-42.

Stamm, C.; I. Friehs, Y.H. Choi, D. Zurakowski, F.X. McGowan, & del Nido P.J. (2003). Cytosolic calcium in the ischemic rabbit heart: assessment by pH- and temperature-adjusted rhod-2 spectrofluorometry. *Cardiovasc Res*. 59:695-704.

Stocum, D.L. (2001). Stem cells in regenerative biology and medicine. *Wound Repair Regen*. 9:429-42.

Strelchenko, N.; O. Verlinsky, V. Kukharenko, & Verlinsky Y. (2004). Morula-derived human embryonic stem cells. *Reprod Biomed Online*. 9:623-9.

Strulovici, Y.; P.L. Leopold, T.P. O'Connor, R.G. Pergolizzi, & Crystal R.G. (2007). Human embryonic stem cells and gene therapy. *Mol Ther*. 15:850-66.

Sun, N.; M.T. Longaker, & Wu J.C. (2010). Human iPS cell-based therapy: considerations before clinical applications. *Cell Cycle*. 9:880-5.

Tagliafico, E.; S. Brunelli, A. Bergamaschi, L. De Angelis, R. Scardigli, D. Galli, R. Battini, P. Bianco, S. Ferrari, G. Cossu, & Ferrari S. (2004). TGFbeta/BMP activate the smooth muscle/bone differentiation programs in mesoangioblasts. *J Cell Sci*. 117:4377-88.

Takahashi, K.; K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, & Yamanaka S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 131:861-72.

Takahashi, K. & Yamanaka S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 126:663-76.

Tamaki, T.; Y. Okada, Y. Uchiyama, K. Tono, M. Masuda, M. Wada, A. Hoshi, T. Ishikawa, & Akatsuka A. (2007). Clonal multipotency of skeletal muscle-derived stem cells between mesodermal and ectodermal lineage. *Stem Cells*. 25:2283-90.

Thomson, J.A.; J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, & Jones J.M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science*. 282:1145-7.

Thomson, J.A. & Marshall V.S. (1998). Primate embryonic stem cells. *Curr Top Dev Biol*. 38:133-65.

Tinsley, J.; N. Deconinck, R. Fisher, D. Kahn, S. Phelps, J.M. Gillis, & Davies K. (1998). Expression of full-length utrophin prevents muscular dystrophy in mdx mice. *Nat Med*. 4:1441-4.

Torrente, Y.; M. Belicchi, C. Marchesi, G. Dantona, F. Cogiamanian, F. Pisati, M. Gavina, R. Giordano, R. Tonlorenzi, G. Fagiolari, C. Lamperti, L. Porretti, R. Lopa, M. Sampaolesi, L. Vicentini, N. Grimoldi, F. Tiberio, V. Songa, P. Baratta, A. Prelle, L. Forzenigo, M. Guglieri, O. Pansarasa, C. Rinaldi, V. Mouly, G.S. Butler-Browne, G.P. Comi, P. Biondetti, M. Moggio, S.M. Gaini, N. Stocchetti, A. Priori, M.G. D'Angelo, A. Turconi, R. Bottinelli, G. Cossu, P. Rebulla, & Bresolin N. (2007). Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. *Cell Transplant*. 16:563-77.

Torrente, Y.; M. Belicchi, M. Sampaolesi, F. Pisati, M. Meregalli, G. D'Antona, R. Tonlorenzi, L. Porretti, M. Gavina, K. Mamchaoui, M.A. Pellegrino, D. Furling, V. Mouly, G.S. Butler-Browne, R. Bottinelli, G. Cossu, & Bresolin N. (2004). Human circulating AC133(+) stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J Clin Invest*. 114:182-95.

Torrente, Y.; G. Camirand, F. Pisati, M. Belicchi, B. Rossi, F. Colombo, M. El Fahime, N.J. Caron, A.C. Issekutz, G. Constantin, J.P. Tremblay, & Bresolin N. (2003a). Identification of a putative pathway for the muscle homing of stem cells in a muscular dystrophy model. *J Cell Biol*. 162:511-20.

Torrente, Y.; E. El Fahime, N.J. Caron, R. Del Bo, M. Belicchi, F. Pisati, J.P. Tremblay, & Bresolin N. (2003b). Tumor necrosis factor-alpha (TNF-alpha) stimulates chemotactic response in mouse myogenic cells. *Cell Transplant*. 12:91-100.

Tremblay, P.; S. Dietrich, M. Mericskay, F.R. Schubert, Z. Li, & Paulin D. (1998). A crucial role for Pax3 in the development of the hypaxial musculature and the long-range migration of muscle precursors. *Dev Biol*. 203:49-61.

Vetrini, F.; R. Tammaro, S. Bondanza, E.M. Surace, A. Auricchio, M. De Luca, A. Ballabio, & Marigo V. (2006). Aberrant splicing in the ocular albinism type 1 gene (OA1/GPR143) is corrected in vitro by morpholino antisense oligonucleotides. *Hum Mutat*. 27:420-6.

Weissman, I.L. 2000. Stem cells: units of development, units of regeneration, and units in evolution. *Cell*. 100:157-68.

Wernig, M.; A. Meissner, R. Foreman, T. Brambrink, M. Ku, K. Hochedlinger, B.E. Bernstein, & Jaenisch R. (2007). In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*. 448:318-24.

Wilmut, I.; N. Beaujean, P.A. de Sousa, A. Dinnyes, T.J. King, L.A. Paterson, D.N. Wells, & Young L.E. (2002). Somatic cell nuclear transfer. *Nature*. 419:583-6.

Woltjen, K.; I.P. Michael, P. Mohseni, R. Desai, M. Mileikovsky, R. Hamalainen, R. Cowling, W. Wang, P. Liu, M. Gertsenstein, K. Kaji, H.K. Sung, & Nagy A. (2009). piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature*. 458:766-70.

Xu, C.; M.S. Inokuma, J. Denham, K. Golds, P. Kundu, J.D. Gold, & Carpenter M.K. (2001). Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol*. 19:971-4.

Yang, X.; S.L. Smith, X.C. Tian, H.A. Lewin, J.P. Renard, & Wakayama T. (2007). Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning. *Nat Genet*. 39:295-302.

Ye, L.; J.C. Chang, C. Lin, X. Sun, J. Yu, & Kan Y.K. (2009). Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases. *Proc Natl Acad Sci U S A*. 106:9826-30.

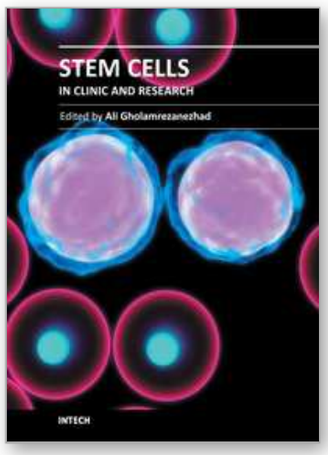
Yu, J.; K. Hu, K. Smuga-Otto, S. Tian, R. Stewart, Slukvin, II, & Thomson J.A. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 324:797-801.

Zhang, X.; P. Stojkovic, S. Przyborski, M. Cooke, L. Armstrong, M. Lako, & Stojkovic M. (2006). Derivation of human embryonic stem cells from developing and arrested embryos. *Stem Cells*. 24:2669-76.

Zhao, Y.; X. Yin, H. Qin, F. Zhu, H. Liu, W. Yang, Q. Zhang, C. Xiang, P. Hou, Z. Song, Y. Liu, J. Yong, P. Zhang, J. Cai, M. Liu, H. Li, Y. Li, X. Qu, K. Cui, W. Zhang, T. Xiang, Y. Wu, Y. Zhao, C. Liu, C. Yu, K. Yuan, J. Lou, M. Ding, & Deng H. (2008). Two supporting factors greatly improve the efficiency of human iPSC generation. *Cell Stem Cell*. 3:475-9.

Zhou, W.; & Freed C.R. (2009). Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells*. 27:2667-74.

Zwi, L.; O. Caspi, G. Arbel, I. Huber, A. Gepstein, I.H. Park, & Gepstein L. (2009). Cardiomyocyte differentiation of human induced pluripotent stem cells. *Circulation*. 120:1513-23.



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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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