Introduction

The last few decades have witnessed major achievements in stem cell (SC) manipulation (Figure 1). This is especially true for hematopoietic stem cells (HSCs) due to the development of SC transplantation several decades ago, and more recently to that of gene therapy (GT) (Figure 2). Lately, SC researchers have made a tremendous breakthrough by artificially inducing cell reprogramming, thus increasing the probability of curing genetic diseases using GT.

However, despite these new attractive concepts and exciting results, artificial modification of genes is also likely to generate unwanted consequences and requires caution. Therefore, safety procedures remain a fundamental issue in the field. To illustrate these progresses and remaining issues, we will present key examples of the use of HSCs and of GT. We will discuss the “duality” of using mesenchymal stem cells (MSCs) and provide perspectives on novel opportunities brought about by a new era of fetal, pluripotent and mature SCs. We will present the development of associated...
therapies, including some aspects linked to the arrival of the highly promising CRISPR-Cas9 technology.

**HSCs: an outstanding model for regenerative medicine**

HSCs are a heterogeneous group of highly plastic adult stem cells (ASCs), which can self-renew and give rise to blood cell lineages. For example, in mice, a single HSC can give rise to epithelial cells of different tissues (28). Similar characteristics of HSCs are maintained in humans (29-31). HSC transplantation (HSCT) has been used since 1958 (32), rendering HSCs the first ASCs successfully used for regenerative medicine to treat leukemic patients. However, as HSCs are very rare, scientists have attempted for years to multiply them without inducing differentiation or to generate them from differentiated cell types, including fibroblasts (33-35). All of these strategies have so far failed to reproduce blood HSC features. However, in the last decade the induced transient expression of six key transcription factors (RUN1T1, HLF, LMO2, PRDM5, PBX1 and ZFP37) finally succeed in producing functional multi-lineages HSCs, as confirmed by their transplantation potential to restore mouse-differentiated blood cells (36). These cells, called induced-HSCs (iHSCs), display significant self-renewal and differentiation potentials at the clonal level. However, expanding the use of iHSCs

**Figure 1** Timeline of stem cell discoveries (1-17).

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1958</td>
<td>First use of a nuclear transfer approach in tadpole (1)</td>
</tr>
<tr>
<td>1960</td>
<td>1961: Demonstration of the existence of stem cells (2)</td>
</tr>
<tr>
<td>1961</td>
<td>1981: First culture of pluripotent cells from mouse embryos (4)</td>
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<tr>
<td>1981</td>
<td>2006: Induced Pluripotent Stem cells (iPSC) from mice (7)</td>
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<tr>
<td>1990</td>
<td>2008:</td>
</tr>
<tr>
<td>1996</td>
<td>- Obtention of iPSC with c-Myc infection (11)</td>
</tr>
<tr>
<td>1998</td>
<td>- Use of iPSC in an animal model of Parkinson’s disease (12)</td>
</tr>
<tr>
<td>2000</td>
<td>1996: Cloning of Dolly (5)</td>
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<tr>
<td>2006</td>
<td>2007:</td>
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<tr>
<td>2007</td>
<td>- Yamanaka’s factors to obtain iPSCs (8)</td>
</tr>
<tr>
<td>2007</td>
<td>- Obtention of human iPSCs from human somatic cells (9)</td>
</tr>
<tr>
<td>2008</td>
<td>- Use of iPSC technology to treat sickle cell disease in mice (10)</td>
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<tr>
<td>2010</td>
<td>2012:</td>
</tr>
<tr>
<td>2012</td>
<td>- First human cell-culture model of Huntington’s disease with iPSCs (14)</td>
</tr>
<tr>
<td>2013</td>
<td>2016: Complete reconstitution of pups starting from skin cells (17)</td>
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<tr>
<td>2017</td>
<td>2017:</td>
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<tr>
<td>2016</td>
<td>2013: First human Stem-cell lines (16)</td>
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</table>
HSC engraftment, including selectin modifications or fucosylation of HSCs (38). Lastly, molecular mechanisms controlling HSC fate determination must be fully deciphered as well as understanding the complexity of HSCs due to their heterogeneity.

**GT: a brief insight into a bright future**

GT consists in transferring *in vivo* or *ex vivo* genetic material into cells through a vector to modify transcriptional expression and correct pathological defects (*Figure 3*). *Ex vivo* GT trials have only been performed using inactivated viral vectors with an impaired replication. First hematopoietic cell-based gene therapies were performed to treat primary immunodeficiencies, like X-linked severe combined immunodeficiency (SCID) (39) and other genetic disorders (23). These trials achieved mild success as some patients developed leukemia following tumorigenic insertion due to the retrovirus. This was shown to occur frequently at the LMO2 gene promoter site (40). LMO1 and 2 belong to the first proto-oncogenes observed in acute lymphoblastic leukemia-type T (ALL-T) (41,42). This transcription factor is of major importance in primary and definitive hematopoiesis during embryonic stages and was therefore also revealed as implicated in ALL. LMO2 translocation is mediated by V(D)J recombinases RAG1 and RAG2 (43). Mouse models expressing the LMO2 oncogene highlighted its importance to cooperate with another transcription factor called Scl/tal1 in the induction of ALL-T. In the case of GT some patients develop an ALL-T, owing to the integration of the vector near LMO2. It was thus speculated that the integration of the vector near LMO2 was instrumental in initiating the oncogenic process (44).

To reduce the risk of developing leukemogenesis, second
generation vectors were created, called “self-inactivating” (SIN) vectors, by inducing a reduction in the adverse transactivation of gene expression. These SIN vectors were then used in a new trial on SCID patients, who displayed no symptoms of leukemia after 4 years (45). If safety is confirmed, GT for SCID patients will become an efficient alternative to haploidentical HSC engraftment (46). New classes of integrating GT vectors are now being developed based on lentiviruses that incorporate SIN safety features to avoid tumorigenic insertion (47).

Gene editing, developed in the last few years, alters DNA sequences using artificially modified nucleases which act as molecular scissors. This process is based on DNA repair mechanisms, namely homology directed repair (HDR) or non-homologous end-joining (NHEJ). Genome editing is limited as high-fidelity HDR only occurs during the G2/S phase, whereas NHEJ induces insertions and deletions. This technique has been used to insert a whole transgene into a defined locus (“safe harbor”) to recover a gene function (45,48). Some studies on HSC-modified zinc finger nucleases (ZFN) were performed to recover the functional expression of IL2RG (mutated in SCID patients) through gene insertion into a safe harbor or downstream of the promoter (49,50). Primitive hematopoietic cells are more sensitive than progenitors to the cytotoxicity of gene targeting procedures and less proficient at performing HDR, because of their quiescence (51). Therefore, in this study, HSC expansion was induced to favor gene editing by HDR. However, despite the high specificity for the IL2R locus, further studies are still required to ensure the absence of off-target changes generated by NHEJ. Determining how to improve HSC transduction rate remains a challenge. Indeed, retroviruses preferentially enter proliferative cells, limiting the number of modified HSCs available for patient engraftment. Importantly, cytokine stimulation can impair bone marrow (BM)-homing and engraftment of CD34+ cells (52). Therefore, a high viral exposure combined to cytokine stimulation could promote mutagenesis and multicopy integration (44). As an alternative strategy, the recent use of a lentiviral vector pseudo-typed with a baboon retroviral envelope glycoprotein resulted in a higher transduction efficiency in quiescent CD34+, at a low concentration without any cytokine stimulation (53).

Altogether, the safety of these different approaches remains questionable. Indeed, NHEJ-mediated gene editing may trigger unwanted modifications. Other techniques are being developed such as genomic insulators that consist of genetic sequences designed to reduce inappropriate gene activation by blocking the ability of enhancers to activate promoters. This seems very promising to reduce insertion mutagenesis. Moreover, a strategy that relies on the insertion of cell type-specific promoters allows a transgene expression restricted to lineage-committed cells. This could be a better strategy to reduce risks of cell transformation when a unique cell lineage is altered, such as in β-thalassemia.
Recent advances in genome editing have been made using a technology derived from the microbial defense system called CRISPR-Cas9. In bacteria, CRISPR-Cas systems provide immunity by incorporating fragments of invading phage and plasmid DNA into CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) loci and using the corresponding CRISPR RNAs to guide the degradation of homologous sequences (54). Cas9 is an endonuclease, associated with CRISPR sequences, able to detect and cleave DNA. It acts as a molecular scissor, for “genome surgery”, enabling the insertion of a sequence of interest. This technology can be applied to correcting genetic mutations, and will certainly become valuable in diverse domains such as understanding the role of genes in biological processes, drug development and pre-clinical use for genome surgery in patients. This apparent “easy-to-use” technology is currently being tested in a phase I clinical trial since 2016 (27). The ease of infecting hematopoietic cells explains why this therapy is initially proposed in monogenic diseases of this tissue. Ongoing GT clinical trials mostly involve cancers then vascular anomalies, and monogenic diseases (55).

Promising potential of MSCs

MSCs definition and properties

MSCs are non-hematopoietic cells of the microenvironment. MSCs are present in most tissues and can be isolated from a variety of different hematopoietic tissues such as bone marrow, as well as from non-hematopoietic adult tissues. The International Society of Cellular Therapy has described the minimum criteria necessary for defining and characterizing multipotent human MSCs in vitro (56,57), such as plastic adherence, morphology, phenotype and potential capacities. Morphologically, MSCs are a heterogeneous population containing cells with a morphology ranging from fibroblast-like to cuboidal (56) and have colony-forming unit-fibroblast (CFU-F) content. Phenotypically, no specific surface antigen marker combination has been defined for MSC populations. However, MSCs express neither markers of hematopoietic lineages (CD34, CD45, glycoporphin A, CD11a, CD14, HLA-DR) nor markers of endothelial lineages (CD11b, CD31), though they express CD29, CD44, CD49, CD51, CD62, CD73, CD90, CD105, CD117, CD166, CD271 and Stro-1 antigens (56). At the functional level, the fact that MSCs can differentiate into bone, cartilage and fat under appropriate stimulatory conditions, represents the major critical requirement to identify putative MSCs population in vitro. Moreover, under certain culture conditions, they can differentiate into dopaminergic neurons, pancreas, cardiac and lung cells, astrocytes and endothelial cells (56,58-60).

An attempt to define the MSC compartment unveiled an initial controversial matter, since cells matching the above-mentioned criteria do not represent a unique cell population but rather a combination of heterogeneous cell types (56,61-63).

Distinct studies revealed the remarkable property of MSCs to spontaneously home to injured sites where they actively participate in tissue regeneration. Interestingly, MSCs have therapeutic benefits due to their ability to act as a trophic factor by delivering many growth factors or bioactive factors. These include antioxidants, pro-angiogenic substances, and cytokines that induce DNA repair by limiting apoptosis and stress responses by mobilizing reparative functions and recruiting immune cells of the recipient (64). These properties render MSCs highly interesting for the treatment of many diseases. MSCs can secrete multiple paracrine growth factors/cytokines involved in inflammation and modulate adaptive immune cells at different levels of the immune response, including in the reprogramming of monocytes/macrophages, in the interference with dendritic cell differentiation, maturation, and function, in modulating natural killer cells, and in T cell activation and suppression of proliferation (64-66). MSCs can support neo-angiogenesis to promote re-vascularization of regenerated tissue (67,68). Indeed, they have been shown to directly promote neo-vessel formation (69,70). Finally, MSCs can support tissue-specific SCs differentiation such as hematopoiesis (71), and have been reported to support HSC maintenance and engraftment (72).

MSCs in clinical applications

MSC-based therapies have been shown to be efficient in preclinical studies in tissue engineering and regenerative medicine for the treatment of several pathologies including cartilage, skin wounding, bone injuries, liver failure (ALF), myocardial infarction, nervous diseases, kidney (AKI) and pulmonary fibrosis (ARDS). To date, there are more than 500 MSC-related clinical research protocols listed in www.clinicaltrials.gov that represent over 660 different conditions and more than 2,000 MSC patients treated
In addition, MSCs could also be used as a delivery platform for therapeutic agents (73). Most of the clinical trials are now in phase I/II and so far, appear to be safe. For example, MSC therapies were recently used in the context of a retinal and optic nerve disease (74), for chronic lung allograft dysfunction (75). It was also used for the regeneration of durable articular cartilage in osteoarthritic knees where no cases of osteogenesis or tumors were observed after 7 years (76). Moreover, MSCs have been used as therapy for chronic obstructive pulmonary disease (COPD) (77) and in congestive heart failure cardiopoietic regenerative therapy (CHART-1) (78).

Technical problems associated with the use of MSCs

Prior to their use in therapies, MSCs need to be isolated from various tissues from multiple origins by various purification techniques. This is a key issue to achieve standardization of MSC isolation protocols. In addition, the heterogeneity of MSCs influences the properties of in vitro expanded MSCs. Next, during in vitro expansion, only a limited number of MSC clones are capable of long-term expansion, and unfortunately, they lose their multipotent potential during this process. MSCs are highly exposed to spontaneous transformation during this proliferation phase in culture. Moreover, the choice of the route of delivery (intravenous injection or intra-arterial local injection) and location of MSCs may affect their efficient trafficking and homing to injured organs. So far, it is very difficult to assess the impact of MSC production and MSC sources on clinical outcome as very few comparable studies have been reported. Therefore, it is important to homogenize and standardize procedures to delimit the conditions and parameters used in the different experiments/trials and choose a unique delivery procedure for further therapeutic consideration.

Different studies have demonstrated the role of MSCs in tumorigenesis. In general, it is believed that MSCs affect tumor growth and invasion through different mechanisms such as the expression of growth factors, increased angiogenesis and metastasis, and/or through modifications in the microenvironment (79,80). MSCs can differentiate into different cell types, such as adipocytes, which develop a pro-tumorigenic activity, or into osteoblasts, which are involved in drug resistance since they can protect leukemic cells from chemotherapy-induced apoptosis via increased engraftment of leukemic cells in the BM (81). However, there is also growing evidence that MSCs increase or inhibit the growth and invasion of tumors through direct or indirect interaction with tumor cells (82). Hence, the mechanisms involved in these processes remain unclear/controversial and there is a real need for further comprehensive studies. Concerning the specific role of MSC alterations in the niche during leukemogenesis, there is no direct evidence demonstrating that an initial lesion in MSCs may play a causative role in human leukemia. For instance, an impaired expression of the ribosome maturation protein SBDS, the ribonuclease Dicer, and the endoribonuclease Drosha, was described in MSCs but not in hematopoietic cells from myelodysplastic syndrome (MDS) patients in comparison with healthy donors (83,84).

The role of the niche, and in particular that of MSCs is the focus of a growing number of studies highlighting that there is a real crosstalk between the niche and leukemic cells (85). Medyouf and colleagues have shown that, in comparison with age-matched counterparts, healthy MSCs significantly enhance MDS CD34+ engraftment in vivo (86), likely owing to the factors differentially expressed between MDS and healthy MSCs such as LIF (87,88), VEGFA (89), IGFBP2 (90), and N-Cadherin (86). Moreover, some alterations in niche cells are sufficient to drive the development of myeloid malignancies in mice (91-93). In contrast, leukemia cells can alter their niche counterpart in genetic mouse models of chronic (CML) and acute myelogenous leukemia (AML) (94).

It was also reported that loss of MSCs, with the associated reduction in CXCL12, is sufficient to accelerate myeloproliferative neoplasm (MPN) progression (95). This phenomenon is reversible and demonstrates that niche transformation represents a major driving force and a requirement for disease progression, and provides a novel, potentially safe therapeutic approach, in which hematopoietic cell-directed therapies were previously shown to be of limited efficacy. Hence, different studies have highlighted that the microenvironment is a possible therapeutic target in AML (96-98).

From embryonic stem cells (ESCs) to induced pluripotent SCs

ESCs

ESCs are constitutive pluripotent cells that originate from the inner cell mass of mammalian blastocysts (5–7 days worldwide.
after fertilization), giving rise to the three germinal layers (endoderm, ectoderm, and mesoderm). ESCs grow in tight colonies, using a feeder layer, and remain undifferentiated indefinitely under defined conditions (99). They spontaneously differentiate into so-called embryonic bodies when cultivated in vitro. In vivo, this spontaneous evolution of ESCs toward differentiation is clearly observed when they are implanted into immunosuppressed mice, forming teratomas, in which cells evolve independently along the differentiation process and grow randomly into the three germinal layers (100). After several passages, chromosome abnormalities appear leading to malignancy (101). This tumorigenic potential is present in a wide range of established tumors, and increases with the number of passages. In addition, ESCs display alterations of their karyotype when cultured for extended periods of time, and highly-passaged cells tend to form less mature tumors. Specific genes affected are linked to cancer and are located on chromosomes involved in culture adaptation. The balance between the generation of enough cells which implies an increased risk of teratoma formation (i.e., potential risk of developing cancer in treated patients) and the use of naïve ESCs that form more mature teratomas (less aggressive, i.e., decrease the level of aggressiveness of cancer cells and their potential transformation) need further investigation. However, if the objective is to prevent all risks of transformation, the technique remains limited in terms of the quantity of cells produced. Altogether it creates a subtly balanced situation for the use of human ESCs in regenerative medicine. Interestingly, neither euploid (abnormality-free cells) nor in vitro cultured ESCs develop teratomas after complete differentiation before transplantation. This discovery could be exploited by exclusively removing tumorigenic-prone cells from differentiated ones, based for example on the use of several fluorescent differentiation markers/probe couples.

Clinical trials have been initiated to explore the potential of ESCs as candidates for regenerative medicine. For instance, ESC therapies are currently tested to treat age-related macular degeneration (ARMD) and spinal cord injury (102).

**Adult SCs**

Other SCs, such as placental, neural or vascular endothelial SCs are of interest in terms of regenerative medicine. Those SCs can be defined as multipotent SCs which give rise to more than one cell type pertaining to a specific cell lineage. Derived from placenta, fetal SCs such as amniotic/chorionic mesenchymal cells and chorionic mesenchymal stromal/trophoblast cells have been shown to differentiate into the three germinal layers [thus including osteogenic, neurogenic, myogenic, adipogenic, pulmonary, cardiac, endothelial, pancreatic or hepatogenic cell types (103)]. To date, placental SC therapeutic applications range from cardiovascular, musculoskeletal, liver, neurological, liver, ocular surface diseases to recent clinical trials investigating their therapeutic use in Crohn's disease, sclerosis, pulmonary sarcoidosis, hematologic disorders, myelodysplasia, graft versus host disease (103,104). Neural stem cells (NSCs), isolated from the adult sub ventricular zone have recently prompted the interest of regenerative medicine researchers, as they display properties of proliferation, self-renewal and differentiation into different mature cell types (105). Several trials are under progress to investigate the therapeutic use of NSCs in damaged central nervous systems, such as strokes, spinal cord injuries, and degenerative diseases (106). Despite promising results in clinical trials, further research on NSCs remains to be conducted, as adult NSCs represent a highly heterogeneous pool of cells, and as the signaling pathways involved in the regulation of NSC properties are still poorly understood (107). Derived from either bone marrow or peripheral blood autologous sources, vascular endothelial SCs (VESCs) are multipotent SCs currently under study in different clinical trials for their potential therapeutic use in hypertension, refractory angina or limb ischemia, thus highlighting the promising potential of VESCs in treating cardiovascular diseases (106). These adult multipotent SCs may lead to the development of autologous regenerative medicine without raising the ethical issues associated with the use of ESCs.

**Induced pluripotent stem cells (iPSCs)**

iPSCs are derived from differentiated somatic cells such as fibroblasts that are reprogrammed following genetic modifications or chemical treatments, to return to a pluripotent SC stage. In 2006, for the first time, Yamanaka and colleagues successfully reprogrammed fully differentiated cells into iPSCs (7) using 4 genes involved in the maintenance of ESC pluripotency, namely Oct3/4, Sox2, c-Myc, and Klf4. In 2007, this technique was reproduced in rats (108,109), monkeys (110), and human fibroblasts (8). iPSCs can be re-directed towards different desired cell types. Importantly, iPSCs can form teratomas and like ESCs can contribute to all cell lineages when
injected into mouse blastocysts, such as cardiac or neural cells (111). The former were obtained by exposing iPSC to activin A and bone morphogenic protein 4 (BMP4) (112). Dopaminergic-like neural cells are obtained by exposing iPSCs to the extrinsic control of microenvironment stromal cell-derived inducing activity (113). Blood and neural immature cells have a predisposition for reprogramming and do so in a more efficient manner, probably due to their epigenetic memory (114-116). The difference between iPSCs and ESCs initially appears to be very slight (Figure 4), especially when considering molecular factors involved in their regulation (99). However, several biological features differ. One of the most remarkable differences in the context of cell therapy resides in the fact that long culture periods do not create detectable alterations in iPSCs.

Several attempts have been made to transfer the reprogramming technology to clinical applications but have been interrupted due to non-clinical issues. In 2014, a Japanese team of surgeons and ophthalmologists successfully implanted cells derived from iPSC on a 70-year-old woman who suffered from an ARMD. A de novo retinal epithelium of only 1.3-by-3 mm$^2$ was successfully implanted (117). Although this procedure did not attempt to restore the vision of the patient, since this is very unlikely, it nevertheless provided an opportunity to monitor side effects, immunogenicity or cancer growth in a human patient. To date it constitutes the first clinical trial on a practical aspect of the iPSCs with a positive outcome, illustrating current hopes in regenerative therapies as evidenced by several other ongoing clinical trials for ARMD.

Figure 4 As embryonic stem cells, inducible pluripotent stem cells can differentiate into all human tissues. iPSC, induced pluripotent stem cell.
treatments using iPSCs or ESCs (118,119). Moreover, new prospects for the treatment of hypertension using iPSCs have recently been described (120). Mechanistically, patient-specific iPSCs currently provide a powerful tool to dissect human single genetic mutation diseases such as familial platelet disorder (121). For example, patient-derived iPSCs are considered to be a new clinical approach to rescue the hemoglobin β gene mutation responsible of β-thalassemia using the CRISPR/Cas9 technology (122).

Unfortunately, frequent teratomas and teratocarcinomas are observed when iPSCs are re-implanted into immunodeficient mice (8). This problem can be partly overcome by introducing Nanog-iPSC, in which the four factors (Klf4, Oct3/4, cMyc, Sox2) are silenced after de-differentiation, whereas the Nanog expression level is maintained. Data using the silencing of the four factors in Nanog-iPSCs demonstrated that these factors are only involved in the induction of pluripotency, but not in the subsequent maintenance of stemness properties. This observation offers opportunities to develop new designs of cancer-free regenerative medicine protocols, switching from a stable expression system (retroviral-based) to a transient one (adenovirus-based), and thus excluding a teratoma-prone environment due to the expression of c-Myc (7).

Differentiation of iPSCs or ESCs into HSCs is currently considered to be another option to generate and experiment on a large number of human HSCs, but the methodologies used produce multipotent progenitors with only a short-term repopulating potential (123). BM stromal-derived iPSCs allow erythroid cell generation with a phenotype closely resembling that of in vivo cells, thereby providing a powerful tool to study erythropoiesis or replacing red blood cell transfusion (124). Recently, monkey iPSC-derived neutrophils have been generated (125). Current efforts are focusing on the development of novel 3D-culture supports for the large-scale culture of inducible HSCs (iHSCs) (126). However, none of these iHSCs have so far been approved for medical use, and a new trial for blood cell generation from iPSCs should start in 2017 (127).

Conclusions & Discussion

Cancer SCs can either originate from true SC transformation or from de-differentiated mature cells (128,129). It is therefore possible that cancer SCs originate from a spontaneous in vivo-in situ reprogramming of either adult cells or de-differentiated mature cells (129,130). In this context, in order to transform an experimental approach into a feasible clinical application, efforts have recently been made to increase the number of re-programmed cells, either by working on the efficiency of the conversion process, to reach a deterministic process (100% efficiency) (131), or by designing simpler culture conditions, independently of the nature of the somatic cells used. The first option has been explored through chromatin remodeling and DNA acetylation mechanisms. Repression of methyl-CpG binding domain protein 3 (MBD3) expression, a subunit of the Mir-2/NuRD complex (an ATP-dependent chromatin remodeling and histone deacetylase complex), was shown to drastically increase the efficiency of the technique, almost reaching 100% within 7 days. Different teams have been able to induce pluripotent SCs using a cocktail of purified proteins derived from the four genes reported by Yamakana, thus avoiding the use of DNA for reprogramming (108,132).

A promising strategy lies in the use of the CRISPR/Cas9 technology, which allows the correction of a genetic mutation, and is also known as “genome surgery”. Following preliminary in vitro and in vivo results, clinical trials are ongoing (27). Today, GT clinical trials are focusing first on cancers, then on vascular anomalies, and on monogenic diseases (55).

In conclusion, the last decades have provided a wealth of novel basic, conceptual and technical knowledge and major advances in regenerative medicine (Figure 5). This upsurge in cell therapy investigations led to renewed perspectives in patient care, with an incredibly broad range of applications in numerous medical fields (Table 1). However, initial experiments and clinical trials also revealed major issues that remain to be addressed. This is especially true regarding the impact of cell-based therapeutic approaches on the risk of cancer development and SC transformation. Both the scientific and medical communities are now aware of these problems and limitations, and the challenges that lie ahead have now been clearly identified. Current investigations and new approaches based on recent advances in the biological understanding of mechanisms controlling SC fate, including epigenetic mechanisms and vectorology, are improving the tools and strategies, to ultimately influence clinical outcome. Therefore, cell therapy is becoming one of the major therapeutic “weapons” to tackle cancer development and to prolong the human lifespan under acceptable conditions, thus drawing us nearer to achieving our wildest dream.
Infusion of MSC

Mesenchymal stem cell

Oct3/4 Sox2 Klf4 cMyc

Fibroblast

Induced Pluripotent Stem Cell

Crispr-Cas9

Therapeutic gene + Viral vector

Hematopoietic stem cell

Figure 5 Graphical abstract.

Table 1 Key examples of current ongoing gene therapy and stem-cell based clinical trials

<table>
<thead>
<tr>
<th>Injected cells/GT</th>
<th>Patients</th>
<th>Stage of clinical trial</th>
<th>Clinicaltrials.gov identifier</th>
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</thead>
<tbody>
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<td>CD34+ cells/SIN GT</td>
<td>X-linked chronic granulomatous disease</td>
<td>I, II</td>
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<td>CD34+ cells/SIN GT</td>
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<td>I, II</td>
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<td>PD-1 knockout CRISPR-Cas9 engineered T cells</td>
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</table>

GT, gene therapy; SIN, self-inactivating; MSC, mesenchymal stem cell; ESC, embryonic stem cell; SC, stem cell; nd, not determined.
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**Footnote**

**Conflicts of Interest:** The authors have no conflicts of interest to declare.

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