CANCER STEM CELL BIOLOGY and CANCER VACCINES
(11)

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Fall-2021

Stem Cell Therapies

- Stem cell-based therapies may be any treatment for a disease or a medical condition that essencially involves the use of any type of viable human stem cells including embryonic stem cells (ESCs), iPSCs and adult stem cells for autologous and allogeneic therapies.

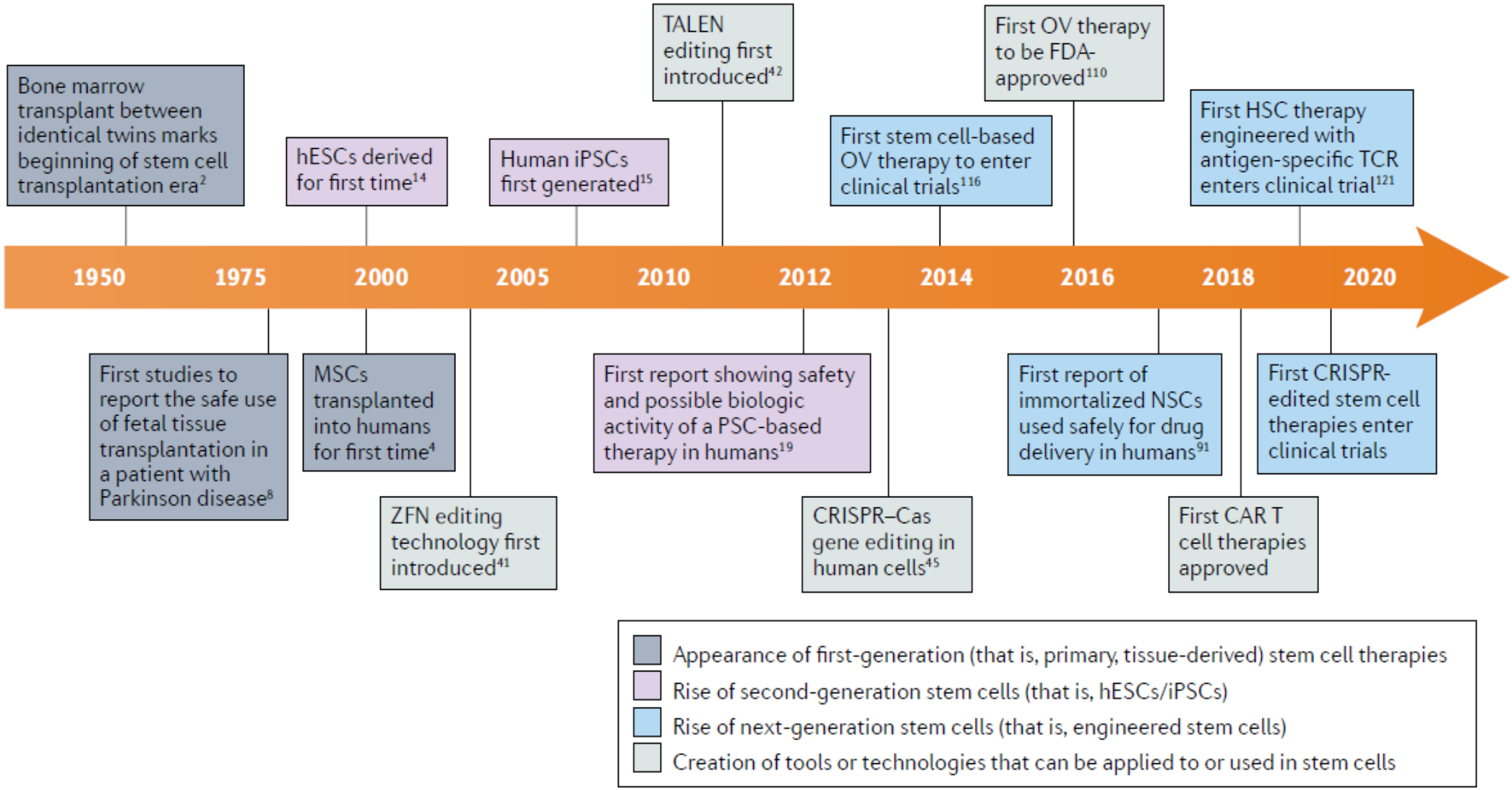
- Stem cells offer the perfect solution when there is a need for tissue and organ transplantation through their ability to differentiate into the specific cell types that are required for repair of diseased tissues.

- However, the complexity of stem cell-based therapies often leads researchers to search for stable, safe and easily accessible stem cells source that has the potential to differentiate into several lineages.

- Thus, it is very important to select the type of stem cells carefully that is suitable for clinical application.

Milestone in Stem Cell Researches

- In 2006, Yamanaka succeeded in generating cells that have the same properties and genetic profile of ESCs.
- This was achieved via the transient overexpression of a cocktail of four transcription factors; OCT4, SOX2 KLF4 and MYC in fully differentiated somatic fibroblast cells and these new cells were called iPSCs.
- A number of challenges still need to be addressed before iPSCs-derived cells can be applied in cell therapies.
- Such challenges include; the detection and removal of incompletely differentiated cells, addressing the genomic and epigenetic alterations in the generated cells and overcoming the tumorigenicity of these cells that could arise on transplantation.

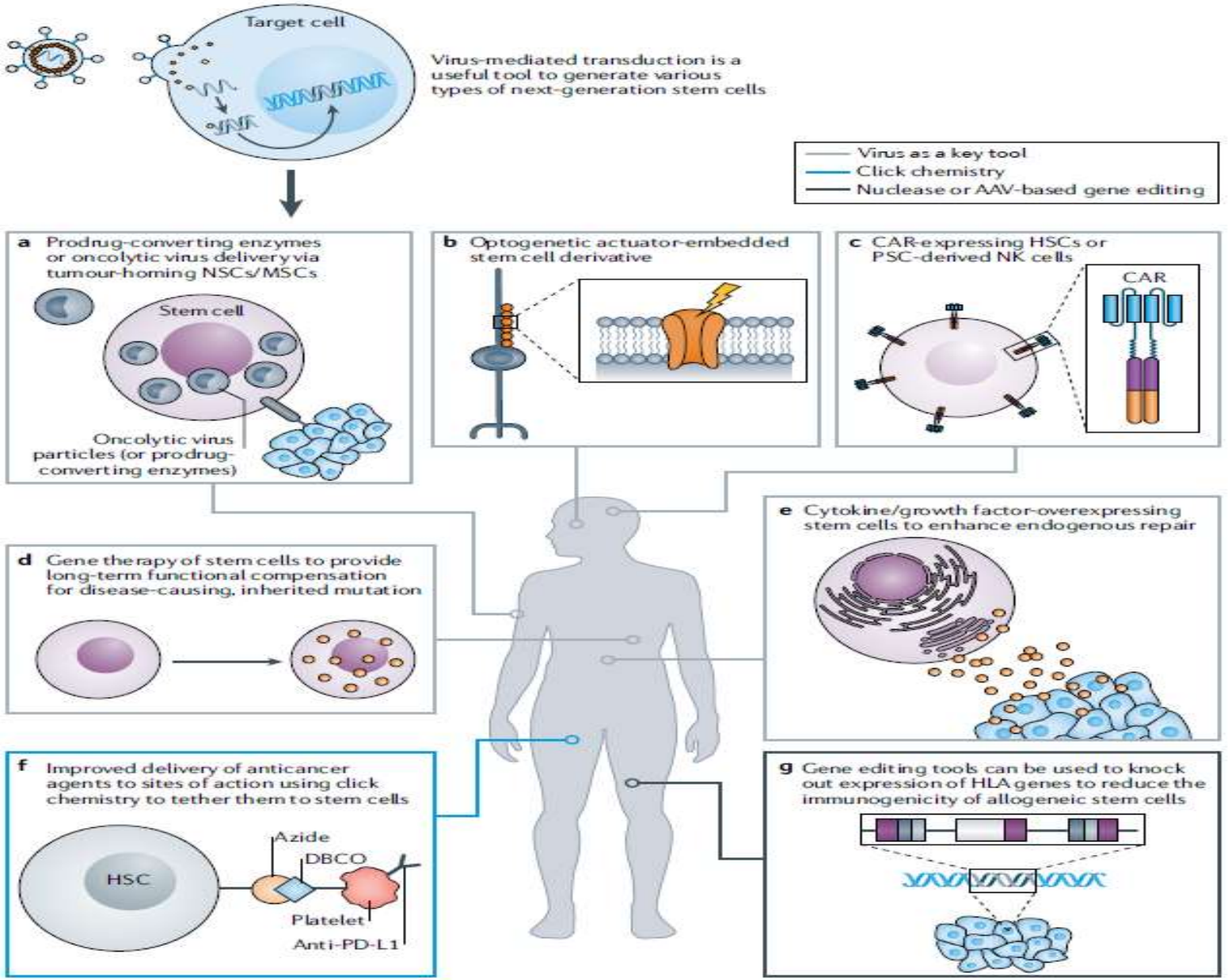


Kimbrel and Lanza, 2020

Stem Cell Based Therapies

1. Virus mediated transduction of stem cells
2. Gene editing tools
3. Other tools
4. Drug delivery vehicles
    a) *Delivery of prodrug- converting enzymes*
    b) *Delivery of apoptosis- inducing agents*
    c) *Delivery of oncolytic viruses*

5. Engineering stem cells as therapeutics



Kimbrel and Lanza, 2020

1. Virus mediated transduction of stem cells

- Virus mediated transduction of exogenous genes (transgenes) can be used to drive the expression of proteins that stem cells would normally not express, such as prodrug converting enzymes, CARs and optogenetic actuators.

- Transgenes may also be used to elevate levels of proteins that are naturally expressed in stem cells, such as growth factors with trophic or reparative effects, or used to express wild- type proteins as a way to functionally compensate for a genetic mutation.

- Commonly used RNA- based retroviruses, such as gammaretrovirus and lentivirus, integrate into the genome, allowing stable, long- term expression of a given transgene. However, integrating viruses carry the risk of inducing insertional mutagenesis.

2. Gene editting tools

- Gene editing is commonly used to create next- generation stem cell therapies. It enables targeted editing of specific genomic loci, which can be used to correct or functionally compensate for genetic mutations, knockout expression of endogenous genes or insert transgene expression cassettes at precise genomic locations.

- Nuclease- based gene editing systems, such as CRISPR–Cas, transcription activator- like effector nucleases (TALENs) and zinc- finger nucleases (ZFNs), are among the most commonly used.

- These nucleases induce double strand breaks (DSBs) at specific sites within the genome. These breaks can be repaired by error- prone non- homologous end joining, which facilitates insertion of a small oligonucleotide sequence or deletion of a small sequence at the DSB site, resulting in disruption of that particular gene.

- Alternatively, by supplying an exogenous template, homology- driven repair can be used to make specific genetic modifications or transgene insertions at that site.

3. Other tools

- Beyond gene therapy and gene editing approaches, other tools are being used to create and improve next-generation stem cells, including optogenetics, chemogenetics and click chemistry:

- **Optogenetics** involves the use of light- responsive proteins to elicit activation of cellular signalling pathways upon exposure to specific wavelengths of light. Optogenetic proteins can therefore be used to spatiotemporally control expression of transgenes, to overcome epigenetic silencing of transgenes or to induce endogenous gene transcription to promote differentiation.

- **Chemogenetics** involves the use of chemicals to control the activity of cells in vivo, which can be achieved through the use of designer receptors exclusively activated by designer drug (DREADDs). The appeal of chemogenetics is the ability to use an orally administered drug to control the activity of transplanted cells that harbour DREADDs, as demonstrated in preclinical models of Parkinson disease and epilepsy.

- Copper- free **click chemistry** conjugation is being used to improve stem cell- based drug targeting to tumour sites and involves the bio- orthogonal reaction between a cyclooctyne, such as dibenzocyclooctyne (DBCO), and an azide- containing surface using a two- step click chemistry approach in stem cells for oncology drug delivery.

4. Drug delivery vehicles

- Stem cells have natural tumour- tropic migratory properties, so packaging anticancer agents within stem cells could provide greater accessibility of these drugs to tumours and/or their metastases, while reducing systemic toxicity.

- Neural stem cells (NSCs) and MSCs have been commonly used for delivery of anticancer agents to tumours, as they are relatively non- immunogenic and migrate in response to tumour- secreted chemoattractants, angiogenic factors and/or inflammatory signals.

- Among the most promising approaches are stem cells loaded with prodrug- converting enzymes, apoptosis-inducing factors or oncolytic viruses (OVs), all of which have reached clinical testing.

Approach	Stem cells	Engineering feature	Cancer	Clinical trial ID	Key refs
Enhanced delivery of chemotherapy	HB1.F3-CD (myc immortalized fetal NSC line)	Cytosine deaminase enzyme to convert 5-FC to 5-FU	High-grade glioma	NCT01172964	87,91
	HB1.F3-CD NSC	Carboxylesterase to convert irinotecan to potent SN-38	High-grade glioma	NCT02192359	93,94
Targeted induction of apoptosis	Autologous bone marrow MSCs	HSV-tk suicide gene	Advanced gastrointestinal adenocarcinoma	NCT02008539	95
	Umbilical cord MSCs	Expression of TRAIL	Stage IIIb/IV metastatic lung adenocarcinoma	NCT03298763	99
Delivery of oncolytic virus	HB1.F3-CD NSCs	CRAd-Survivin-pk7 oncolytic adenovirus	Malignant gliomas	NCT03072134	115
	Adipose MSCs	Thyroidal sodium iodide symporter-expressing oncolytic measles virus	Recurrent ovarian cancer	NCT02068794	116
Delivery of checkpoint inhibitor	HSCs	Conjugated platelets with attached PD-1 antibodies	Leukaemia	NA (preclinical stage)	82

5-FC, 5-fluorocytosine; 5-FU, 5-fluorouracil; HB1.F3-CD, HB1.F3 cell line expressing cytosine deaminase; HSC, haematopoietic stem cell; HSV-tk, herpes simplex virus thymidine kinase; MSC, mesenchymal stem cell; NA, not available; NSC, neural stem cell; PD-1, programmed cell death protein 1; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.

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a) Delivery of prodrug- converting enzymes

- If targeted to a tumour, prodrug- converting enzymes can facilitate the local conversion of a non- toxic prodrug to its active cytotoxic form, potentially reducing serious adverse effects compared with systemic administration of the active cytotoxic agent.

- Retroviruses have been used to genetically modify NSCs and MSCs to express the prodrug- converting enzyme cytosine deaminase.

5FC ⟶ 5FU

- One of the challenges of this approach is the need to generate large quantities of cells without altering their tumour- tropic properties.

b) Delivery of apoptosis- inducing agents

- The tumour tropic nature of stem cells is also being leveraged to deliver apoptosis- inducing drugs to highly aggressive brain tumours, non- small- cell lung cancer and pancreatic cancer.

- In a small phase I trial (NCT02008539), autologous bone marrow- derived MSCs genetically modified to express the suicide gene encoding herpes simplex virus thymidine kinase (HSV- tk) were safe and well-tolerated in patients with advanced gastrointestinal adenocarcinoma. HSV- tk converts the prodrug ganciclovir to the toxic ganciclovir triphosphate, which inhibits DNA polymerization and induces apoptosis in actively dividing cells.

- The toxic metabolite is also taken up by neighbouring cells through a bystander effect, which induces further cell death within a tumour.

- Another apoptosis- inducing approach involves overexpression of tumour necrosis factor- related apoptosis-inducing ligand (TRAIL) in stem cells. TRAIL is a secreted cytokine that binds to death receptors on cancer cells and induces their apoptosis.

c) Delivery of oncolytic viruses

- OVs exploit tumour associated changes in cell surface markers and cellular proliferation pathways to selectively kill cancer cells.

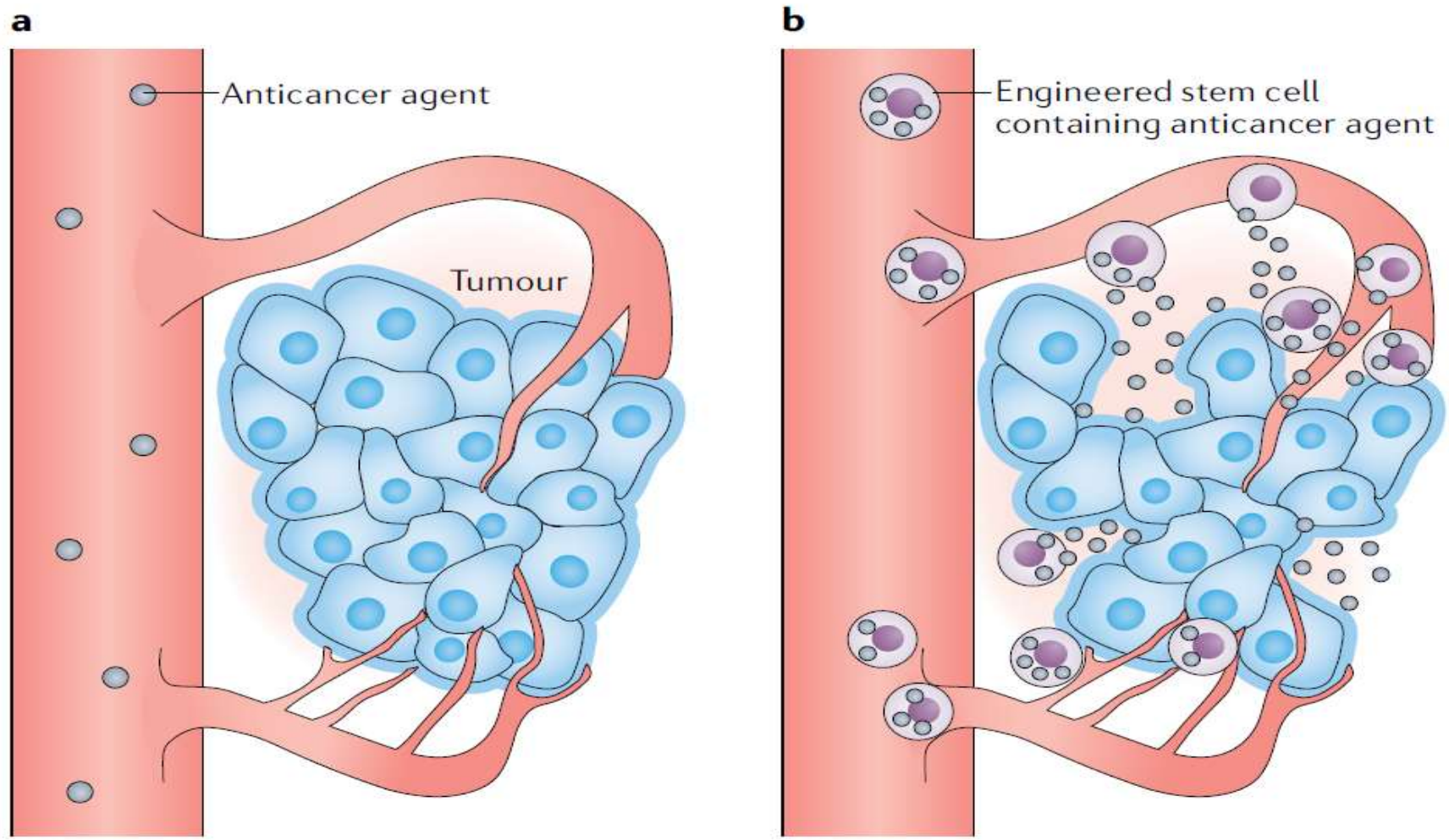
- OVs have been studied for many years and were recently validated as an anticancer modality with the approval of T- VEC by the FDA in 2015 for the treatment of melanoma with metastatic lesions in the skin and lymph nodes.

- Various viruses, including adenovirus, measles virus, HSV and vaccinia virus, can be engineered as OVs to augment their immune activation properties against cancer cells and/or to enhance their infectivity.

- OVs may initiate an interferon response, triggering antiviral immune mechanisms and activation of natural killer cells (NK cells), dendritic cells and cytotoxic T cells, which may limit their effectiveness.

- Neutralizing antiviral antibodies may limit the effectiveness of OVs, particularly upon repeat administration, and certain virus types may have limited biodistribution and/or difficulty in crossing the blood–brain barrier.

- Packaging of OVs within stem cells can be used as a **‘Trojan- horse’** delivery approach to shield the Ovs from physiological mechanisms that may limit their biodistribution and, thus, effectiveness.
- In a melanoma brain metastasis model, MSCs loaded with oncolytic HSV homed to various metastatic areas upon intracarotid artery injection, whereas the unshielded oncolytic HSV administered alone did not.
- The increased distribution afforded by the MSC- packaged oncolytic HSV correlated with an increase in survival of the metastatic brain tumour- bearing mice.



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5. Engineering stem cells as therapeutics

- Next- generation stem cells can also serve as therapeutic agents themselves, in various applications such as (i) immuno- oncology, (ii) tissue repair and (iii) inherited disease.

i. In immuno- oncology, next- generation HSCs can provide long- term antigen- specific immunity through their multilineage engraftment capabilities, whereas differentiation of next- generation PSCs may provide off- the- shelf antigen- specific immunotherapies.

ii. For use in tissue repair, stem cells are being engineered to overexpress neurotrophic factors, anti-inflammatory cytokines or angiogenic factors to facilitate the healing and recovery of tissues damaged by injury or disease.

iii. For treating inherited diseases, next- generation stem cells are being used to provide long- term enzyme replacement, to correct or override the effects of disease- causing mutations.

Stem Cell Therapy in the Illnesses

- Currently, some stem cell-based therapies utilizing adult stem cells are clinically available and mainly include bone marrow transplants of hematopoietic stem cells and skin grafts for severe burns.

- To date, there are more than 3,000 trials involving the use of adult stem cells registered in WHO International Clinical Trials Registry. Additionally, initial trials involving the new and appealing iPSCs based therapies are also registered.

Stem Cell Therapy in the Illnesses

A) Neurodegenerative diseases

The main target is to treat the disorders like Parkinson's, Alzheimer's, ALS and MS in addition to detain progression of these diseases.

Generation of neural cells from stem cells have been succsessfully applied *in vitro* studuies.

Stem Cell Therapy in the Illnesses

A) Neurodegenarative diseases

- **Parkinson's disease:**

  Midbrain dopaminergic neurons are lost.

  Dopaminergic-like neurons from ESC/iPSC

  In August 2019 Yamanaka initiated the first approved clinical trial using iPSC.

- **MS:**

  Neurodegenerative and inflammatory autoimmune disease of central nervous system

  2014, registered clinical trial, company Celgene, multiple centered,but cellular treatment did not improve the MS condition

  Replacing damaged neural tissue with neural cells derived from iPSC.

- **Spinal cord injury:**

  Japan has recently given approval of using stem cells in spinal cord injury (first governmental approval)

  Injection of stem cells isolated from patients' bone marrow to regain lost sensation and mobility

Stem Cell Therapy in the Illnesses

B) Ocular diseases

- Huge number of clinical trials for stem cell-based therapies because eyes are immune privileged sites
- Allogeneic ESC lines
- The first clinical trial to implement the use autologous iPSCs-derived retinal cells was in Japan which followed the new regulatory laws issued in 2014 by Japan's government to regulate regenerative medicine applications
- Macular degeneration using iPSCs-generated retinal cell sheet
- iPSCs-based autologous transplantation was safe and feasible

Stem Cell Therapy in the Illnesses

C) Diabetes

- Pancreatic beta cells are destructed in type 1 diabetes mellitus, because of disorders in the immune system while in type 2 insulin insufficiency is caused by failure of the beta-cell to normally produce insulin.
- In both cases the affected cell is the beta cell, and since the pancreas does not efficiently regenerate islets from endogenous adult stem cells, other cell sources were tested.
- Pluripotent stem cells (PSCs) are considered the cells of choice for beta cell replacement strategies
- The company ViaCyte in California recently initiated a phase I/II trial (NCT02239354) in 2014 in collaboration with Harvard University
- The preclinical studies demonstrated successful glycemic correction and the devices were successfully retrieved after 174 days and contained viable insulin-producing cells

Stem Cell Therapy in the Illnesses

D) Dentistry

Stem cells have been successfully isolated from human teet and were studied to test their ability to regenerate dental structures and periodontal tissues. MSCs were reported to be successfully isolated from dental tissues like dental pull of permanent and deciduous teeth, periodontal ligament, apical papilla and dental follicle.

Dental stem cells demonstrated superior abilities in immunomodulation properties either through cell to cell interaction or via a paracrine effect. Stem cells of non-dental origin were also suggested for dental tissue and bone regeneration.

- Dental pulp regeneration
- Periodontal tissue regeneration
- Regeneration of mandibular bony defects

Stem Cell Banking

The ability to bank autologous stem cells at their most potent state for later use is an essential adjuvant to stem cell-based therapies.

- iPSCs theoretically possess the ability to proliferate unlimitedly which pose them as an attractive source for use in cell-based therapies. Unlike adult stem cells iPSCs ability to propagate does not decrease with time. Recently, California Institute for Regenerative Medicine (CIRM) has an iPSCs store to provide researchers with variable iPSCs cell lines in order to accelerate stem cell treatments through studying genetic variation and disease modeling.
- Umbilical cord is immediately cryopreserved after birth; which permits stem cells to be successfully stored and ready for use in cell-based therapies for incurable diseases of a given individuals.
- Stem cells of human exfoliated deciduous teeth (SHEDs) have the capacity to differentiate into further cell types than the rest of the adult stem cells. Moreover, procedures involving the isolation and cryopreservation of these cells are un-complicated and not aggressive. *SHEDs is the insured autologous transplant which avoids the possibility of immune rejection.* Contrary to cord blood stem cells, *SHEDs have the ability to differentiate into connective tissues, neural and dental tissues.*
- MSCs can be stored too.

Regulatory Principles of Stem Cell Therapies

- The Food and Drug Administration (FDA) has released regulatory guidelines to ensure that these treatments are safe and effective
- In 2014, a radical regulatory reform in Japan occurred with the passing of two new laws that permitted conditional approval of cell-based treatments following early phase clinical trials on the condition that clinical safety data are provided from at least ten patients.
- The treatments that acquired conditional approval include those targeting; spinal-cord injury, cardiac disease and limb ischemia.
- Regulatory authorities are now demanding application of standardization and safety regulations protocols for cellular products, which include the use of Xeno-free culture media, recombinant growth factors in addition to “Good Manufacturing Practice” (GMP) culture supplies.

Regulatory Principles of Stem Cell Therapies

- ESCs are far superior regarding their potency; however, their derivation requires destruction human embryos. True, the discovery of iPSCs overcame this concern.
- iPSCs themselves currently face another ethical controversy of their own which addresses their unlimited capacity of differentiation with concerns that these cells could one day be applied in human cloning. The use of iPSCs in therapy is still considered a high-risk treatment modality, since transplantation of these cells could induce tumor formation.

✓ *developing optimized protocols to ensure their safety*

✓ *developing global clinical-grade iPSCs cell lines before these cells are available for clinical use*

Regulatory Principles of Stem Cell Therapies

- MSCs, these cells have been universally considered safe, however continuous monitoring and prolonged follow-up should be the focus of future research to avoid the possibility of tumor formation after treatments.

- It could be postulated that one of the most challenging ethical issues faced in the field of stem cell-based therapies at the moment, is:

the increasing number of clinics offering unproven stem cell-based treatments!

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