PÉCSI TUDOMÁNYEGYETEM
ÁLTALÁNOS ORVOSTUDOMÁNYI KAR
Immunológiai és Biotechnológiai Intézet

Biological therapies

Gene and stem cell therapies

Olasz Katalin
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OUTLINE

1. GENE THERAPY

2. STEM CELLS AND STEM CELL-BASED THERAPIES

Historical background

- **Gene therapy** is the treatment of a disease through transferring genetic material, either DNA or RNA, into cells of the patients
- **1972**: **Gene Therapy for Human Genetic Disease? Review**
    1. restored the ability to synthesize the enzyme thymidine kinase to thymidine kinase-deficient mouse cells by infection with herpes simplex virus (Munyon et al.)
    2. treatment of fibroblasts from patients with galactosemia with exogenous DNA caused increased activity of a missing enzyme (Merril et al.)
- **1987: Gene therapy may have future role in cancer treatment?**
- Half-a-century's intense work, gene therapy has achieved significant success, with over three dozens of gene therapies officially approved as clinically used drugs

Genetic medicines: treatment strategies for hereditary disorders

- More than **1,800** known monogenic **hereditary disorders**

- Development of '**genetic medicines**' → 3 dozens of approved gene therapies
- **<u>Strategies</u>**:
  - use of somatic stem cells
  - gene transfer
  - RNA modification

O'Connor & Crystal, Nature Rev Gen, 2006

Gene therapy approved for clinical uses 1.

Trade name	Target disease	Company	Approved country/area	Vector	References
Vitravene[a]	CMV retinitis	Ionis Pharmaceuticals	USA (1998), Europe (1999)	Oligonucleotide	[80]
Gendicine	Head and neck cancer	Shenzhen SiBiono GeneTech	China (2003)	Adenovirus	[20]
Oncorine	Nasopharyngeal cancer	Shanghai Sunway Biotech	China (2005)	Adenovirus	[22]
Rexin-G	Soft tissue sarcoma, osteosarcoma, and pancreatic cancer	Epeius Biotechnologies	Philippine (2007)	Retrovirus	[81]
Neovasculgen	Atherosclerotic peripheral arterial disease	Human Stem Cell Institute	Russia (2011)	Plasmid	[82]
Glybera[b]	Familial lipoprotein lipase deficiency	UniQure	Europe (2012)	AAV	[83]
Kynamro	Hypercholesterolemia	Genzyme	USA (2013)	Oligonucleotide	[84]
Imlygic	Melanoma	Amgen	USA, Europe (2015)	HSV	[23]
Exondys 51	Duchenne muscular atrophy	Sarepta Therapeutics	USA (2016)	Oligonucleotide	[85]
Zalmoxis	Restores immune system after HSCT	MolMed	Europe (2016)	Retrovirus	[86]
Strimvelis	Severe combined immunodeficiency	GlaxoSmithKline	Europe (2016)	Retrovirus	[87]
Spinraza	Spinal muscular atrophy	Biogen	USA (2016), Europe (2017)	Oligonucleotide	[36]
Invossa	Knee osteoarthritis	Kolon Life Science	Korea (2017)	Retrovirus	[88]
Yescarta	Large B-cell lymphoma	Kite Pharma	Europe, USA (2017)	Retrovirus	[89]

Gene therapy approved for clinical uses 2.

Trade name	Target disease	Company	Approved country/area	Vector	References
Kymriah	B-cell precursor acute lymphoblastic leukemia and large B-cell lymphoma	Novartis Pharmaceuticals	USA (2017), Europe (2018)	Lentivirus	[90]
Luxturna	Retinal dystrophy	Spark Therapeutics	USA (2017), Europe (2018)	AAV	[91]
Onpattro	Amyloidosis	Alnylam Pharmaceuticals	Europe, USA (2018)	Lipid-siRNA	[92]
Tegsedi	Amyloidosis	Ionis Pharmaceuticals and Akcea Therapeutics	Europe, Canada, USA(2018)	Oligonucleotide	[93]
Waylivra	Familial chylomicronemia syndrome	Ionis Pharmaceuticals and Akcea Therapeutics	Europe (2019)	Oligonucleotide	[94]
Collategene	Critical limb ischemia	AnGes MG	Japan (2019)	Plasmid	[95]
Zynteglo	β-thalassemia	Bluebird Bio	Europe (2019)	Lentivirus	[96]
Zolgensma	Spinal muscular atrophy	AveXis	USA (2019) Japan, Europe (2020)	AAV	[97]

[a]Withdrawn from the European market in 2002 and USA market in 2006 due to decreased demand
[b]Withdrawn from the market in 2017 due to low demand

In 2025 approved new gene therapies:

Therapy	***Disease***	***Type***
Zevaskyn	RDEB	Gene-corrected skin graft
Waskyra	Wiskott-Aldrich Syndrome	Lentiviral HSC therapy
Itvisma	SMA (expanded use)	AAV gene replacement

Main groups of viral vectors

Table 1 | **The main groups of viral vectors**

Vector	Genetic material	Packaging capacity	Tropism	Inflammatory potential	Vector genome forms	Main limitations	Main advantages
Enveloped							
Retrovirus	RNA	8 kb	Dividing cells only	Low	Integrated	Only transduces dividing cells; integration might induce oncogenesis in some applications	Persistent gene transfer in dividing cells
Lentivirus	RNA	8 kb	Broad	Low	Integrated	Integration might induce oncogenesis in some applications	Persistent gene transfer in most tissues
HSV-1	dsDNA	40 kb* 150 kb‡	Strong for neurons	High	Episomal	Inflammatory; transient transgene expression in cells other than neurons	Large packaging capacity; strong tropism for neurons
Non-enveloped							
AAV	ssDNA	<5 kb	Broad, with the possible exception of haematopoietic cells	Low	Episomal (>90%) Integrated (<10%)	Small packaging capacity	Non-inflammatory; non-pathogenic
Adenovirus	dsDNA	8 kb* 30 kb§	Broad	High	Episomal	Capsid mediates a potent inflammatory response	Extremely efficient transduction of most tissues

*Replication defective. ‡Amplicon. §Helper dependent. AAV, adeno-associated viral vector; dsDNA, double-stranded DNA; HSV-1, herpes simplex virus-1; ssDNA, single-stranded DNA.

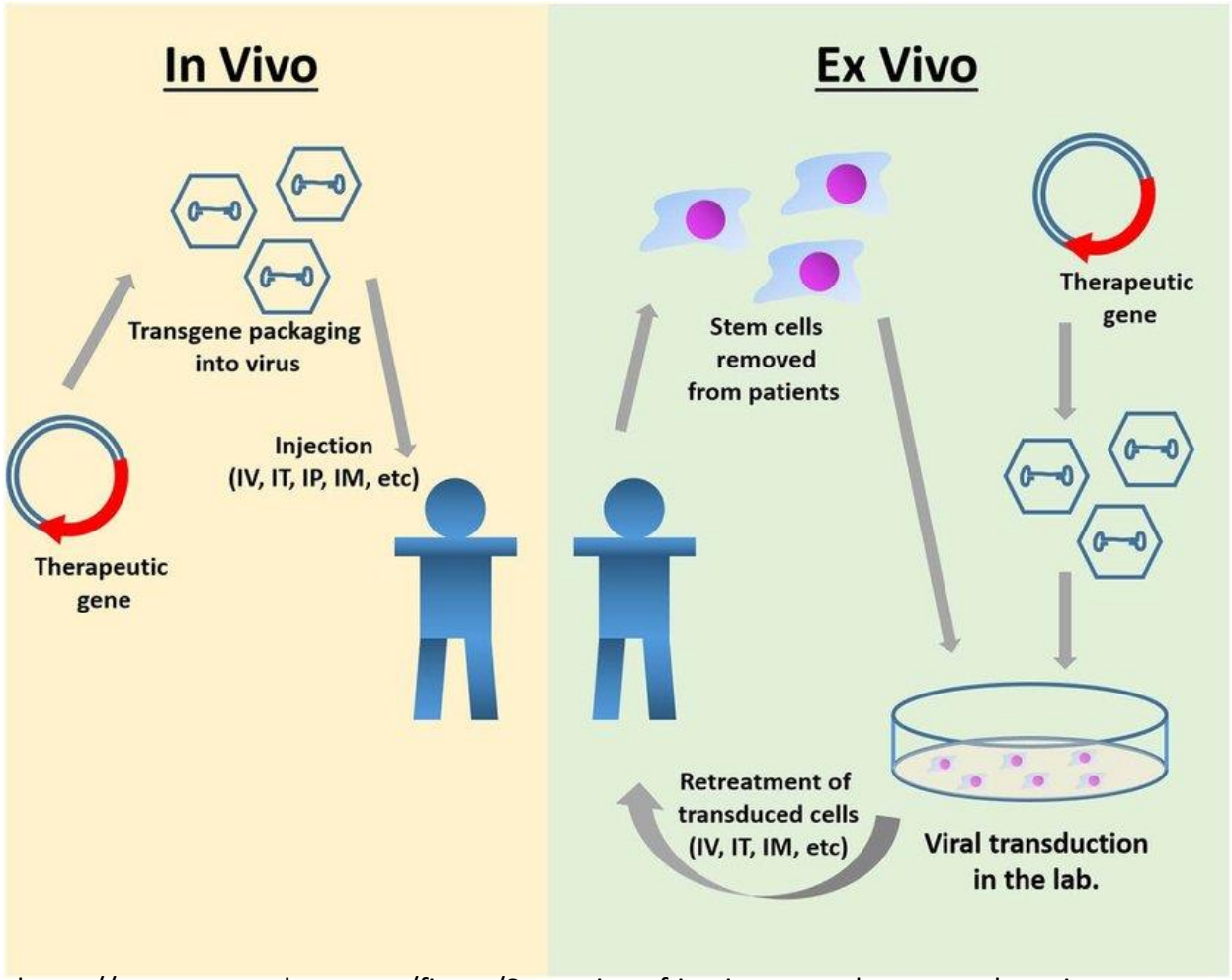
Possible ways of act

1. Enabling expression of the transferred gene
2. Inhibiting the expression of a target gene
3. Modifying a target gene

Strategies in gene therapy 1.

In vivo gene delivery

- Vector carrying the expression cassette is administered directly to the patient
- **Main vector:** Adeno-associated viruses (AAV) -non-integrating vectors=DNA is not integrated into the genome
- **Advantage:** reduces risks of insertional mutagenesis
- **Disadvantage**: limits long-term expression
- **Treatment of:** familial lipoprotein lipase deficiency (Glybera), retinal dystrophy (Luxturna), and **spinal muscular atrophy** (Zolgensma)



https://www.researchgate.net/figure/Strategies-of-in-vivo-gene-therapy-and-ex-vivo-gene-therapy-In-vivo-gene-therapy-on-the_fig1_322970469

Ex vivo gene delivery

- **Removal of target cells** from the individual
- **Genetic modification** in a laboratory
- **Transplantation back** into the patient
- **Vectors**: γ-retroviruses and lentiviruses
- **Treatment of** adenosine deaminase-associated **SCID** (Strimvelis), **β-thalassemia** (Zynteglo), and **large B-cell lymphoma** (Yescarta and Kymriah)

Strategies in gene therapy 2.

Gene addition

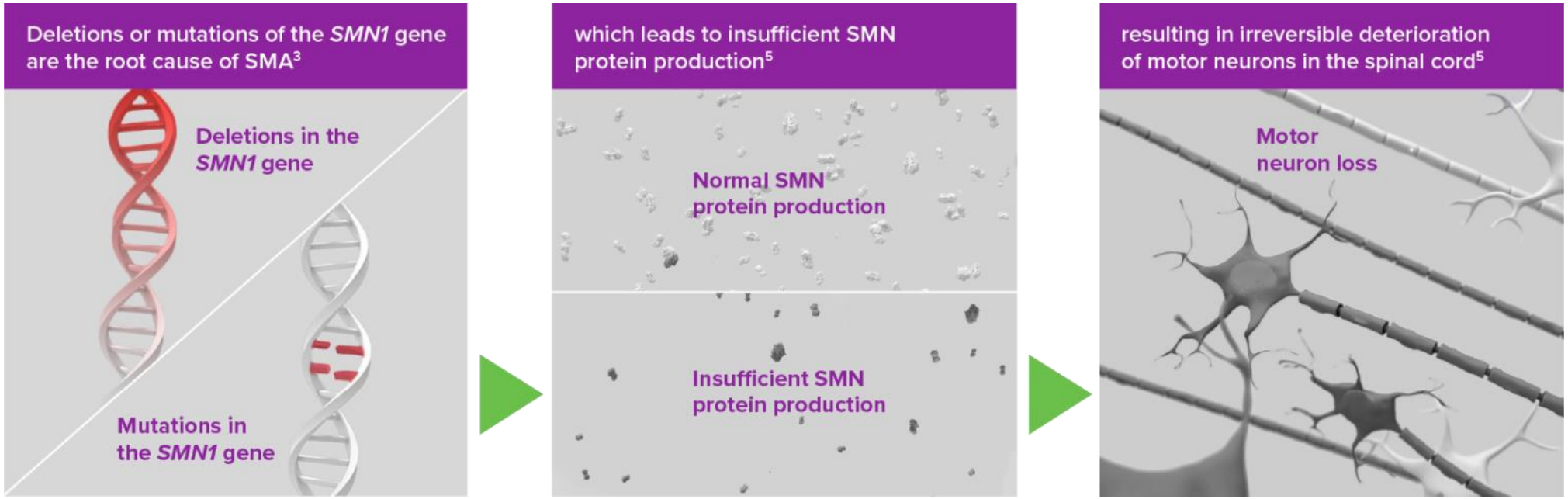
- Normal copy of a specific defective **gene is delivered** into the target cells and **restores the function** of the defective gene
- **RNA interference** (RNAi), which inhibits the expression of the defective gene ➡ **small inhibitory RNAs (siRNAs)** are delivered (non-viral) or **short hairpin RNA (shRNA)**-encoding gene that will ultimately generate siRNAs is delivered (viral)
- **siRNAs base-pair with the mRNA**s of the defective gene and promotes their **degradation**
- **Treatment of**: **spinal muscular atrophy (Spinraza),** familial hypercholesterolemia (Kynamro)

Genome editing

- Possible **removal or correction** of the defective genes in the genome **permanently**
- **Nuclease-encoding gene** is delivered into target cells **via AAV vectors**
- **Nuclease** could be delivered into target cells **in the form of mRNA or protein with** the help of **nanoparticles or lipids**
- **Nuclease** ➡ DNA double-strand breaks (DSBs) at specific genomic loci ➡ disruption or correction of the defective gene
- Insert a corrective gene into a “safe” genomic loci
- **no genome editing-based gene therapy approved for clinical uses**

What is SMA?

- **Spinal muscular atrophy (SMA)** is a rare genetic disease caused by the **deletion or mutation of the *survival motor neuron 1* (*SMN1*) gene**.
- The *SMN1* gene produces **survival motor neuron (SMN) protein** that is critical for normal function of motor neurons.
- Patients with SMA have an **insufficient amount of SMN protein**, which leads to **permanent loss of motor neurons**. Untreated, SMA Type 1 is the number one genetic cause of infant death.



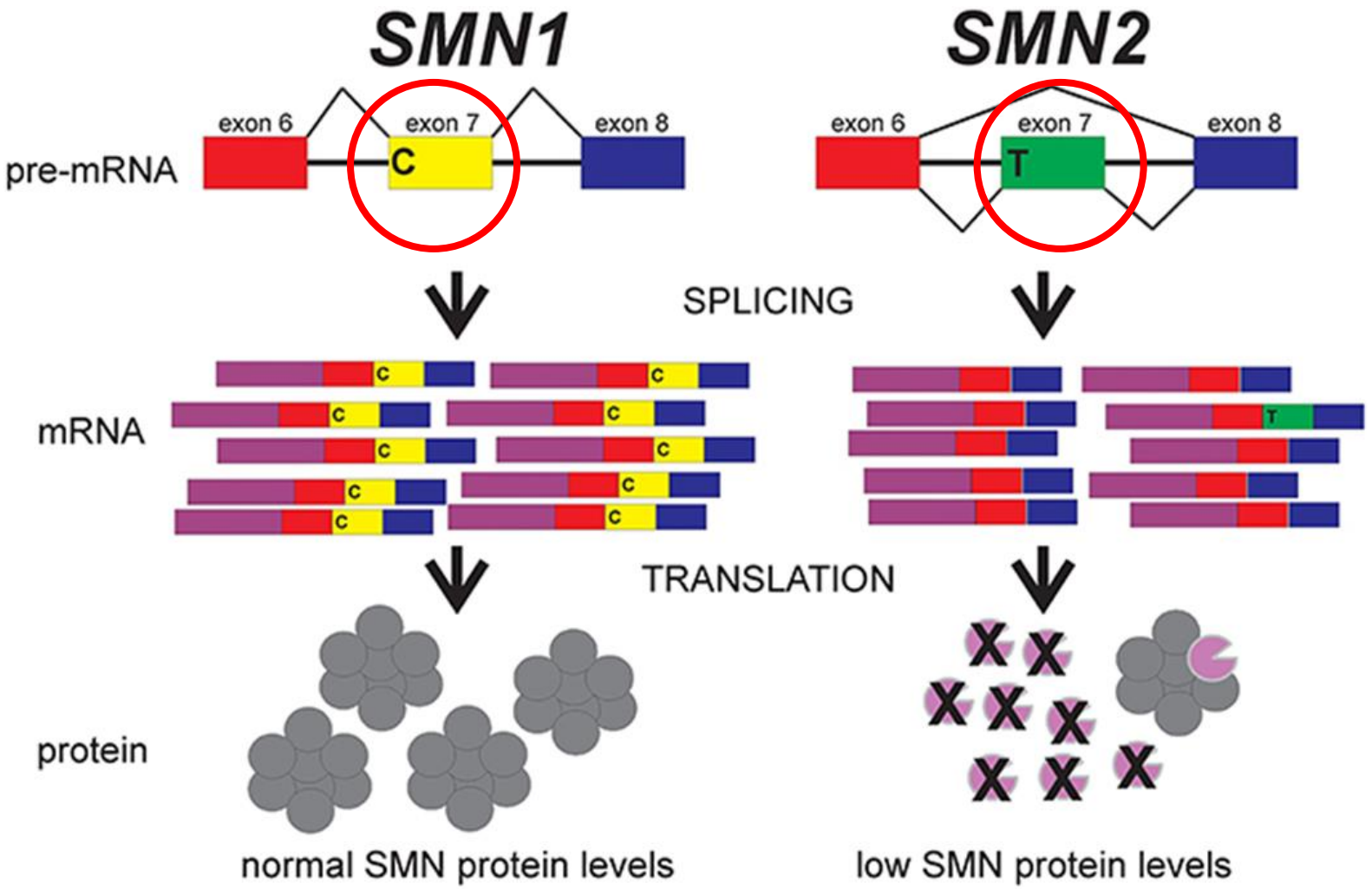
https://www.zolgensma-hcp.com/understanding-sma/what-is-sma/

Types of SMA

https://www.zolgensma-hcp.com/understanding-sma/what-is-sma/

SMA Type	Type 1	Type 2	Type 3
SMN1[5]	Nearly all patients with SMA, regardless of type, will have bi-allelic deletions or mutations of *SMN1*		
SMN2 copy number[5,10]	1-3	2-3	3-4
Incidence rate[11]	~60%	~27%	~12%
Age of onset[12]	0-6 months	6-18 months	>18 months
Maximal motor milestones achieved[12]	Never achieve sitting	Sit but never walk	Stand and walk
Key clinical features[3,4]	Severe hypotonia, respiratory insufficiency, poor feeding and head control	Scoliosis, unable to walk independently, proximal weakness	Proximal weakness, may lose ability to walk over time

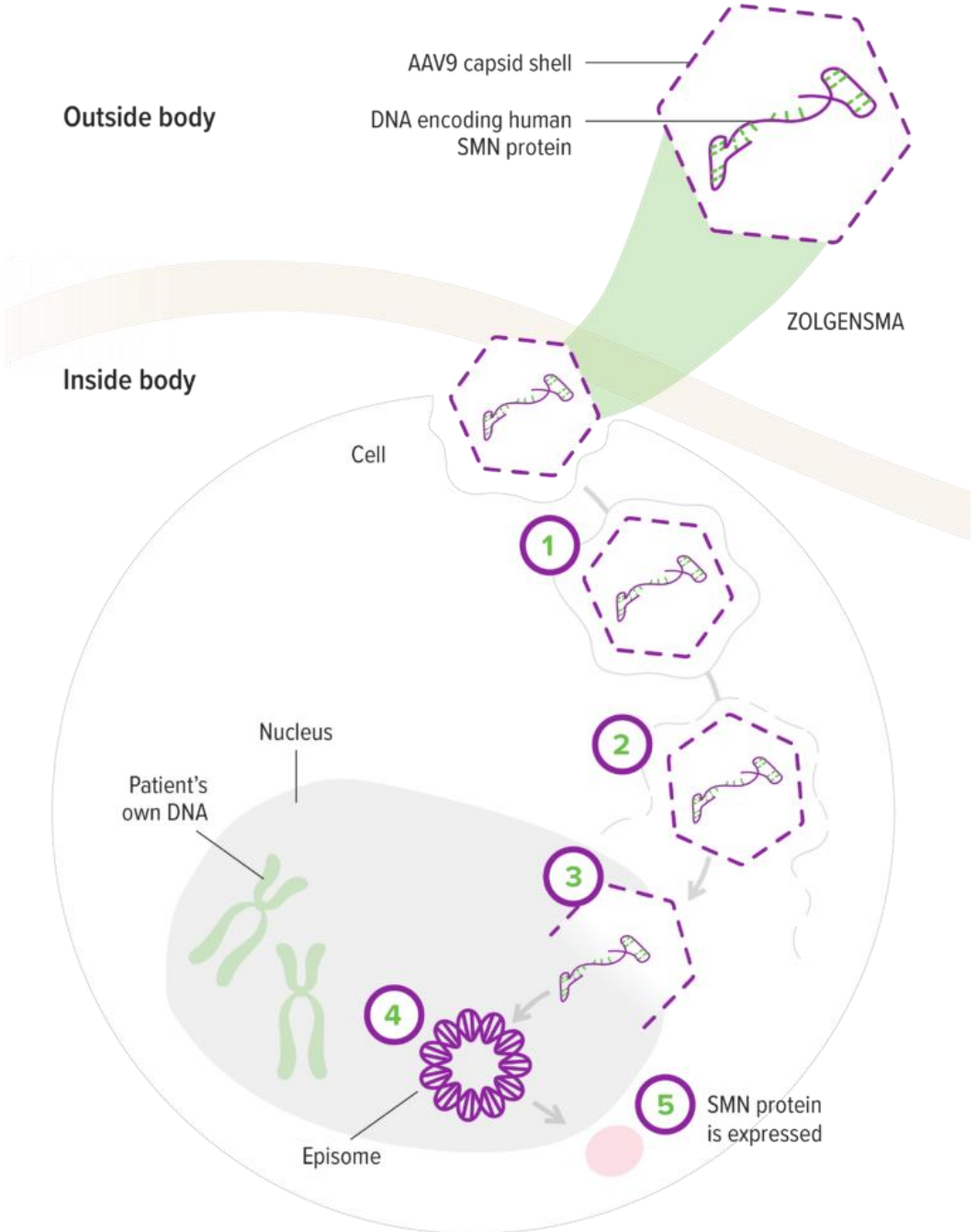
SMN1 and SMN2 genes



- In healthy individuals, the **SMN1 gene** is functional and produces **stable SMN protein**.
- The **SMN2** allele is the **disease-modifying gene** because of a single nucleotide difference(C-T transition) in exon 7 that results in alternative processing of its mRNA and editing out of exon 7.

How ZOLGENSMA works?

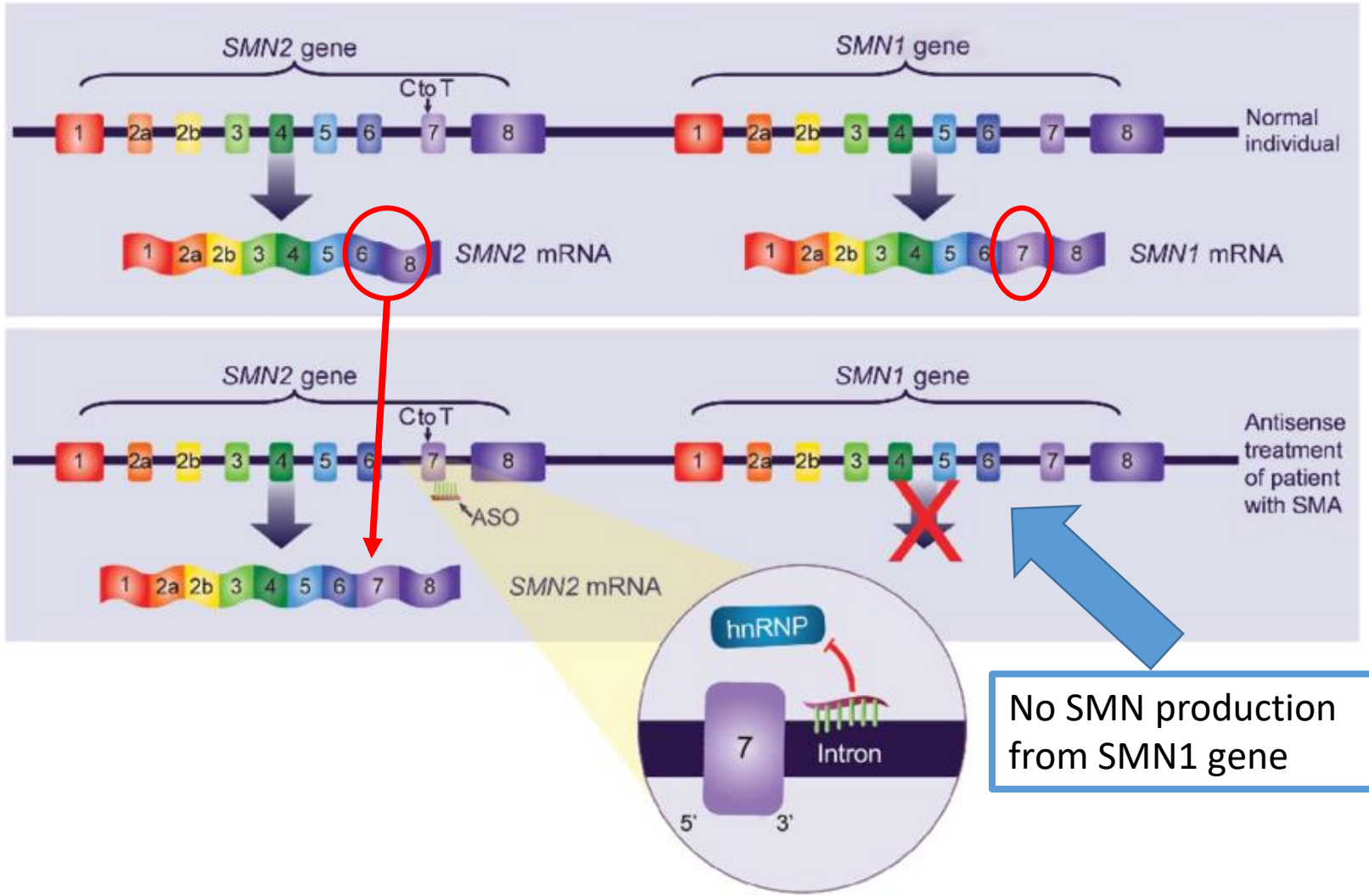
1. The **AAV9 (Adeno-associated virus) vector** enters motor neuron cells
2. The AAV9 vector delivers the *SMN* gene to the cell nucleus
3. The *SMN* gene is introduced to target cells as recombinant, self-complementary DNA
4. The **self-complementary ends** form a **circular episome** that can persist in the nucleus of motor neuron cells. (These cells are nondividing)
5. This results in **rapid activation and continuous expression of the *SMN* gene**



https://www.zolgensma-hcp.com/understanding-sma/what-is-sma/

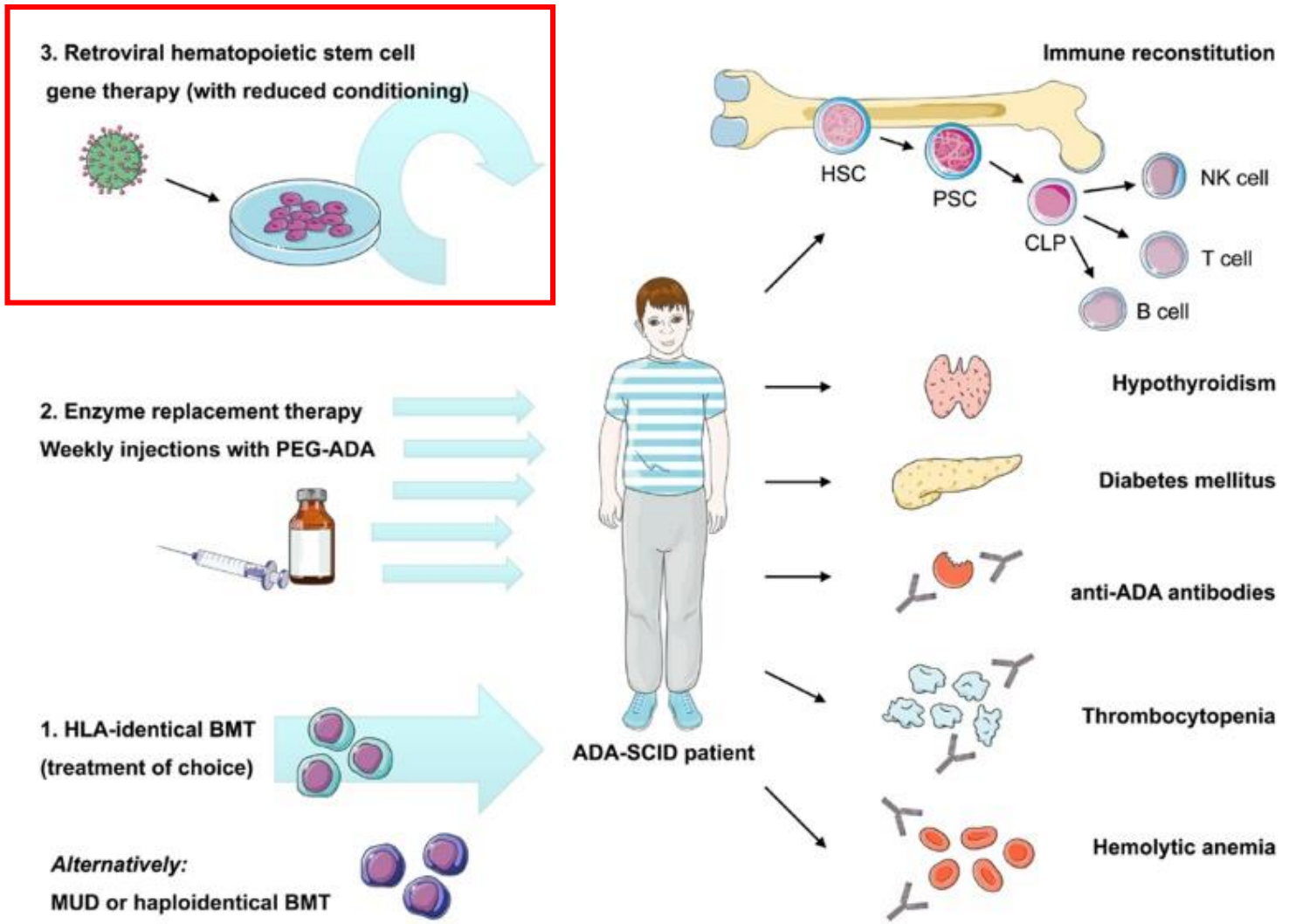
SMA treatment with Spinraza (Nusinersen)

- A **C to T transition in exon 7 of the SMN2 gene** leads to the **exclusion of exon 7** from the final transcript, producing an **unstable and nonfunctional SMN protein**.
- **Nusinersen** targeting the SMN2 gene, and **allows the inclusion of exon 7 in the mRNA reading frame**. Nusinersen hybridizes to ISS-N1 and blocks its splicing of exon 7 allowing for the **inclusion of exon 7 in the mRNA** and high **protein production from the SMN2 gene**.



ADA-SCID

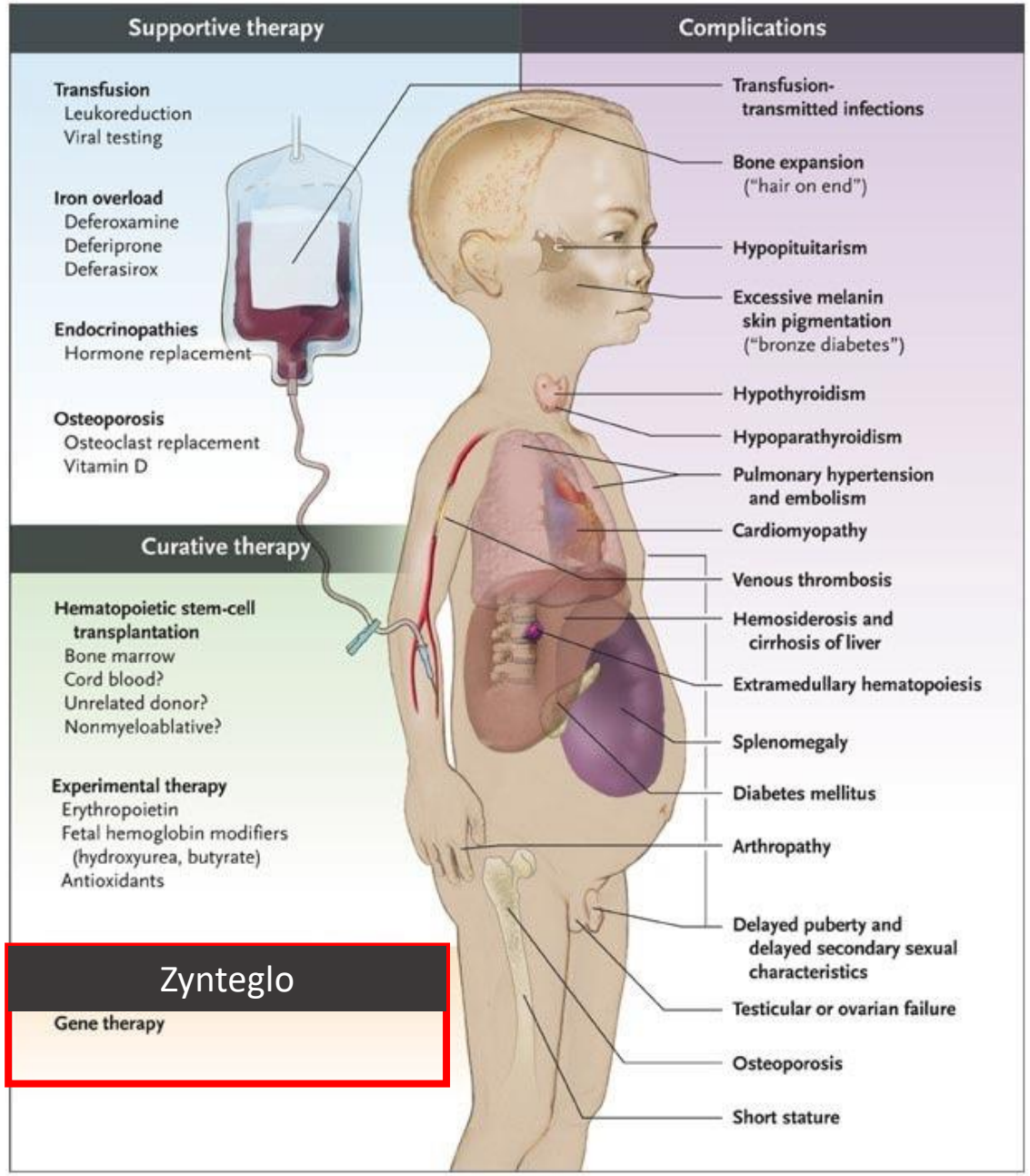
- Adenosine deaminase deficiency is the second-most prevalent form (approximately 20%) of SCID
- Lymphopenia, severely impaired cellular and humoral immune function, autoimmune manifestations
- **<u>Available therapies</u>**:
  - bone marrow transplantation (BMT)
  - enzyme replacement therapy with bovine ADA (PEG-ADA)
  - **hematopoietic stem cell gene therapy** (HSC-GT) (combination of gene and stem cell therapy)



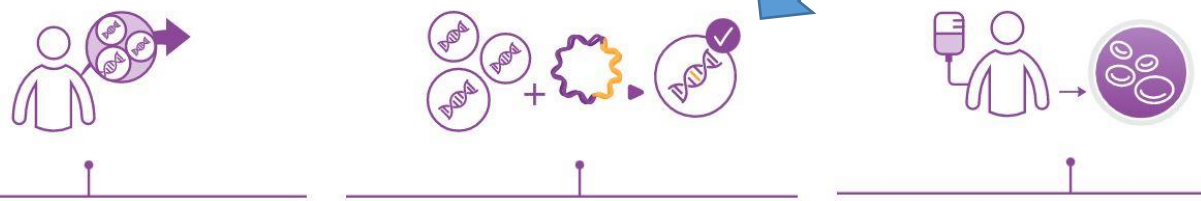
https://www.frontiersin.org/files/Articles/29368/fimmu-03-00265-r2/image_m/fimmu-03-00265-g002.jpg

β-Thalassemia

- Inherited disorder: mutations in the hemoglobin beta (HBB) gene
- Decreased production of β-globin chains
- Affects multiple organs
- Minor, intermediate and major forms
- **ZYNTEGLO** uses a **lentiviral vector (LVV)**. LVV uses HIV's natural ability to deliver genes into a cell but does not include the genes that cause HIV infection (combination of gene and stem cell therapy)



Genetically modified („corrected") stem cells



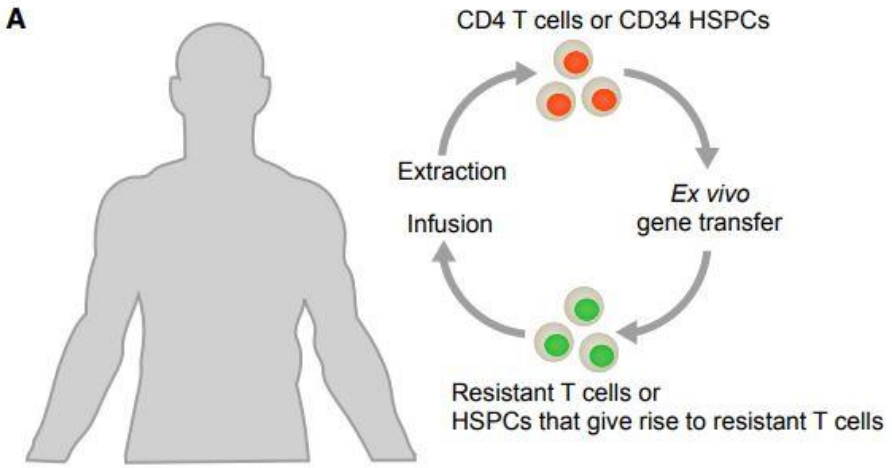
https://www.nejm.org/doi/full/10.1056/nejmra050436

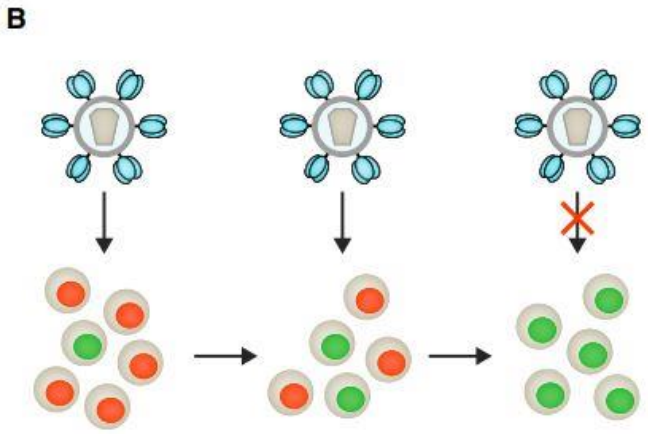
Strategies in gene therapy 3.

Acquired disease

- First **anti-cancer** gene therapy Gendicine (2003) for treatment of head and neck cancer through delivering a **tumor suppressor gene *p53*** into patients
- **Oncolytic virus-based gene therapy** Oncorine (2005) for nasopharyngeal cancer (adenovirus that selectively propagates in p53-deficient tumor cells)
- **CAR-T gene therapy**: treatment of **cancer** (2017. Yescarta and Kymriah were approved for treatment of large B-cell lymphoma and B-cell precursor acute lymphoblastic leukemia)
- Possible treatment for **acquired immune deficiency syndrome (AIDS)**: homozygous 32-bp deletion in the *CCR5* gene provides resistance against HIV-1 entry

Gene therapy could cure HIV?





Inevitable for the immune system

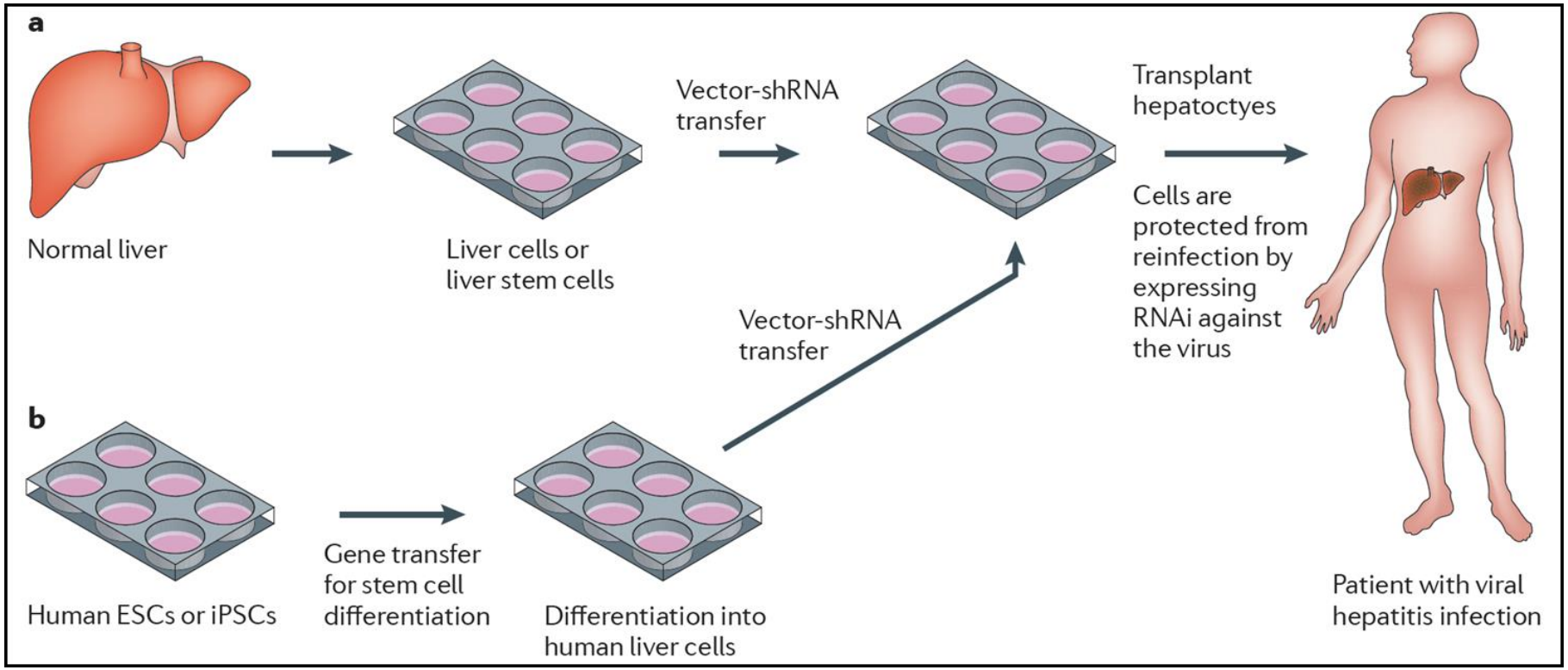
CD4: receptor
CXCR4 or **CCR5** function as a co-receptor

Dispensable

Reduction of CCR5 on the surface of HIV target cells

- **CD4+ T cells or CD34+ HSPCs** (not infected by HIV) **are extracted** from a patient, **genetically modified ex vivo** to express one or multiple antiviral genes, and **infused into the same patient**.
- Gene-modified and unmodified cells coexist in the patient. Ideally, the gene-modified HIV target cells would have a **survival advantage** over unmodified cells and replace the unmodified HIV target cell population over time, resulting in **an immune system that is resistant to HIV**.
- **Approaches**: retain CCR5 inside gene-modified cells, Ribozymes and shRNAs target CCR5 mRNA, CRISPR-associated protein-9 nuclease (Cas9) introduce mutations into the CCR5 gene.

Gene therapy for viral heptitis infection



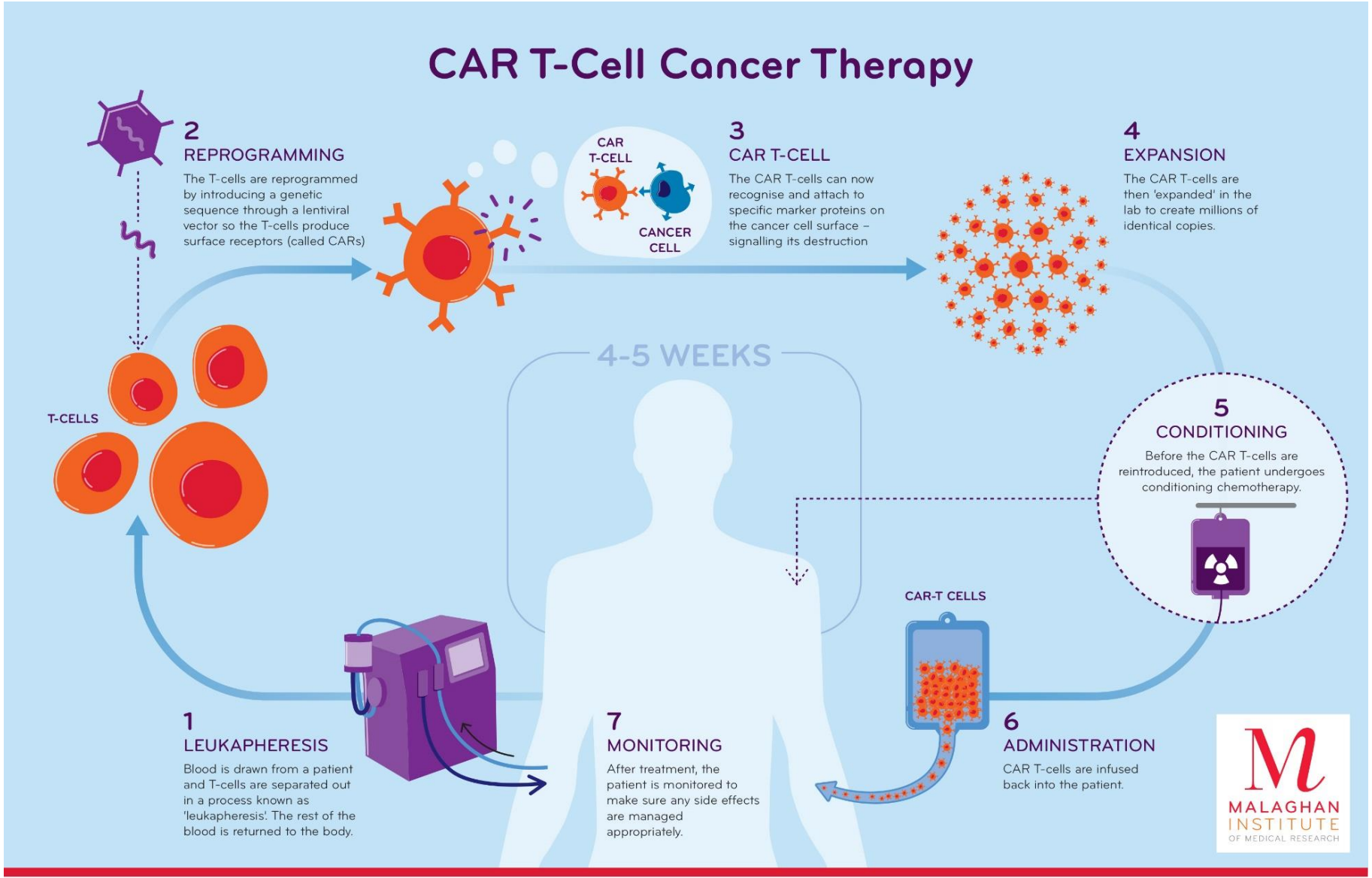
Possibilties:

1. Hepatocellular transplantation of mature hepatocytes
2. Hepatocytes-derived from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs)
3. Gene transfer of a vector encoding RNAi (short hairpin RNAs) directed against the virus would make the transplanted cells **resistant or 'immune' to reinfection**.

Likely to become reinfected by the hepatitis virus

Kay MA, Nature Rev Gen, 2011

Chimeric Antigen Receptor T-cell therapy



Strategies in gene therapy 4.

Somatic gene therapy

- The various strategies discussed above all belong to somatic gene therapy, which is **applied to somatic cells** that need to be corrected or modified.
- Not prevent transmission of the defective gene from parents to children

Germline gene therapy

- Applied to **gametes or preimplantation embryos**
- Promise to prevent passage of genetic disease not only to the patient's children, but to all future generations
- At present, there is **no GGT approved to be used clinically yet**

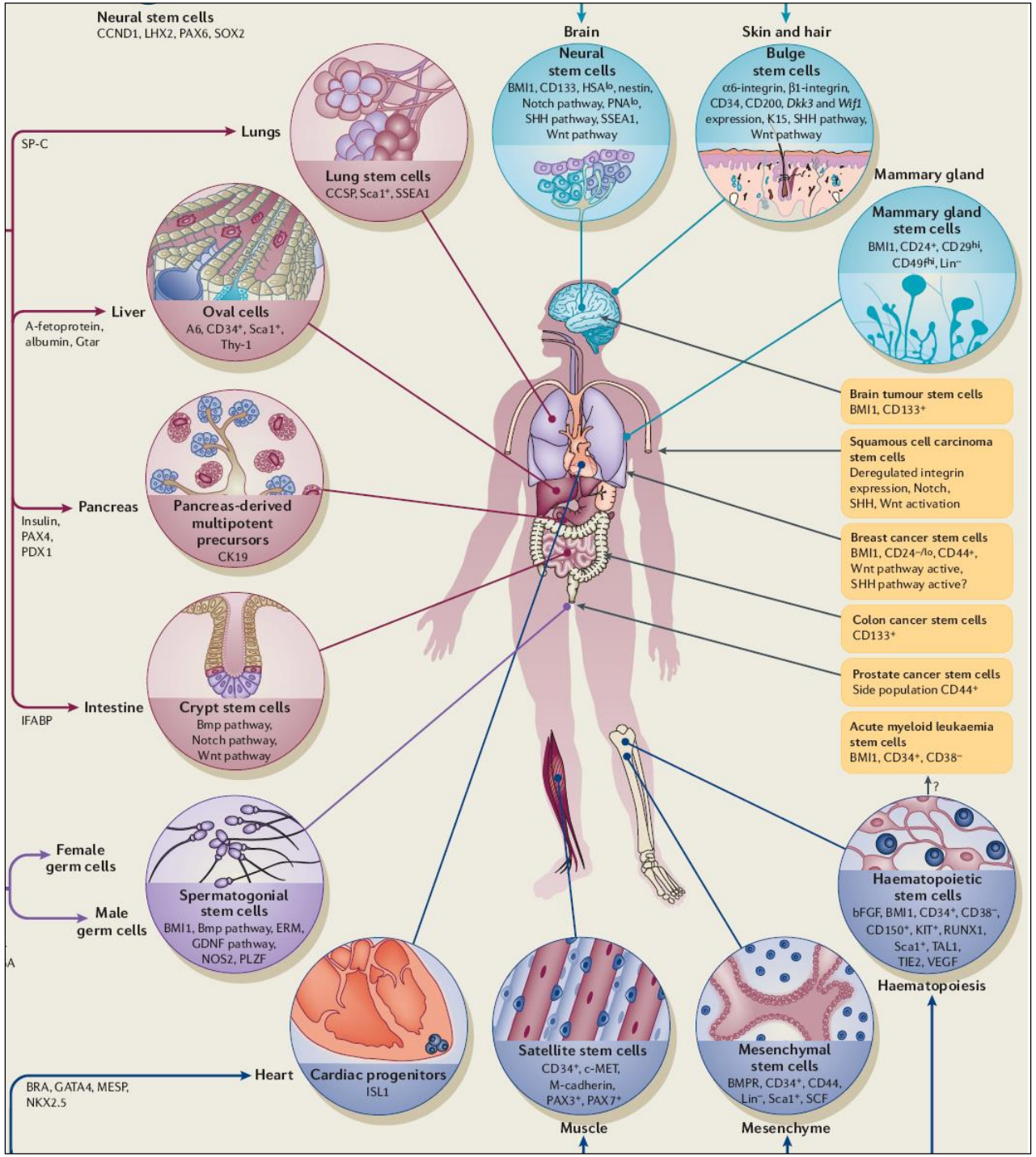
https://link.springer.com/article/10.1007/s11010-020-03834-3

OUTLINE

1. GENE THERAPY

2. STEM CELLS AND STEM CELL-BASED THERAPIES

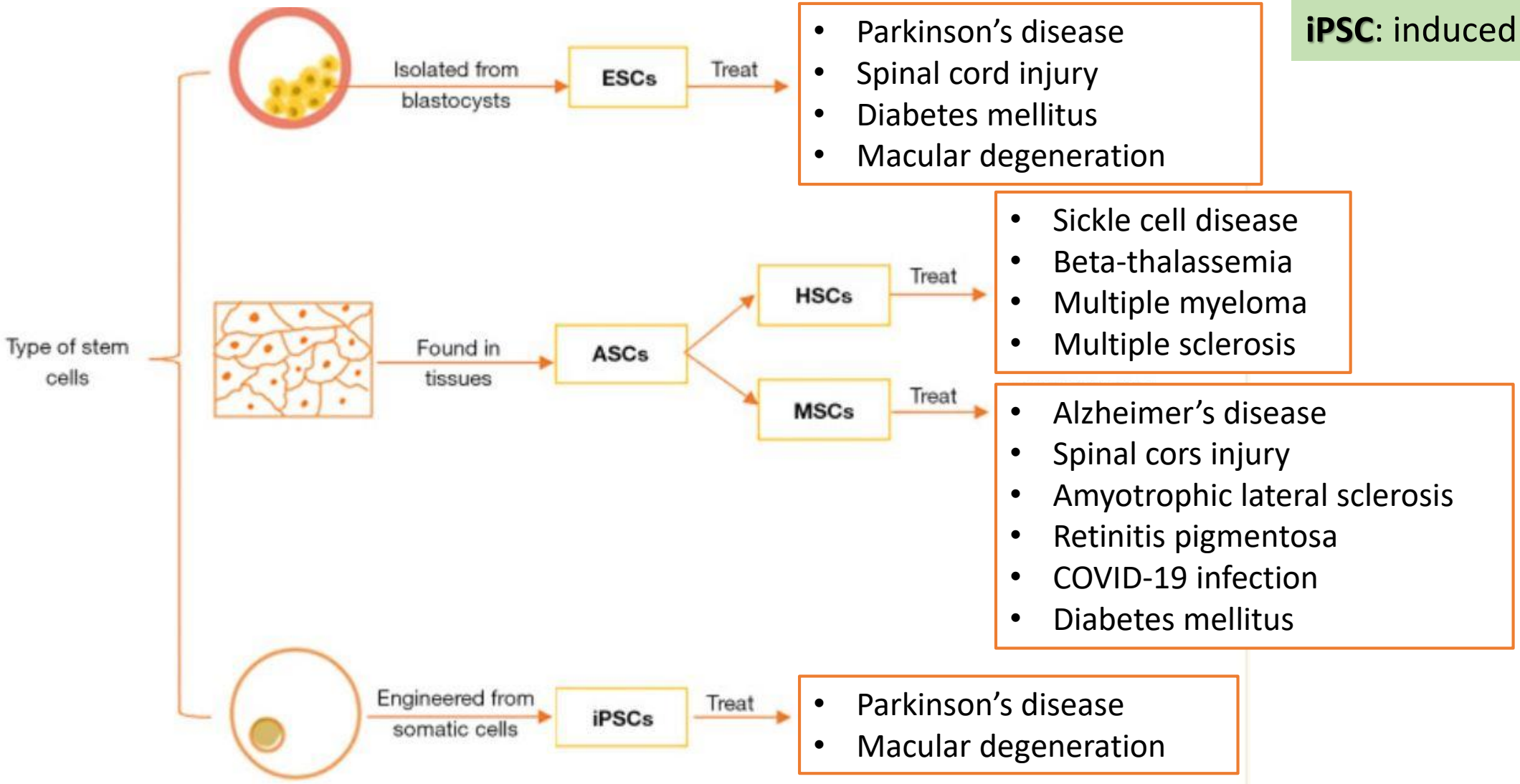
Stem cells in various organs and tissues



Watt & Eggan, Nature Rev Cancer, 2006

Types of stem cells

ESC: Embryonic Stem Cell
ASC: Adult Stem Cell
HSC: Haemotopetic Stem Cell
MSC: Mesenchymal Stem Cell
iPSC: induced Progenitor Cell



- Parkinson's disease
- Spinal cord injury
- Diabetes mellitus
- Macular degeneration

- Sickle cell disease
- Beta-thalassemia
- Multiple myeloma
- Multiple sclerosis

- Alzheimer's disease
- Spinal cors injury
- Amyotrophic lateral sclerosis
- Retinitis pigmentosa
- COVID-19 infection
- Diabetes mellitus

- Parkinson's disease
- Macular degeneration

Characteristics and differences between the three major types of stem cells

Characteristics	ESCs	ASCs	iPSCs
Source	Inner cell mass of blastocyst (27)	Niches in body tissues (41)	Adult somatic cells (10)
Potency	Pluripotent (27) Able to differentiate into all three germ layers; ectoderm, endoderm and mesoderm Able to differentiate into all types of cells	Multipotent (42) Able to differentiate into multiple specialised cell types based on tissue niche such as MSCs, HSCs, NSCs	Pluripotent (10) Able to differentiate into all three germ layers; ectoderm, endoderm and mesoderm Able to differentiate into all types of cells
Risk of immune rejection	High (38)	Low (38)	Low (38)
Risk of teratoma formation	High (38)	Low (38)	High (45)
Ethical issues	Present due to the use of embryos and potential use for human cloning (45)	Absent (38)	Present due to the potential use for human cloning (46)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9552054/

The milestones achieved in the development of stem cell therapy

1956	First stem cell therapy: HSCs to treat leukaemia in identical twins (6)
1981	First *in vitro* cultivation of mouse ESCs (7)
1995	First clinical use of mesenchymal stem cells (MSCs) to investigate feasibility of transfusion (8)
1998	First *in vitro* cultivation of human ESCs (9)
2006	Introduction of iPSCs (10)
2010	First stem cell therapy: ESCs to treat spinal cord injury (SCI) (11)
2014	First stem cell therapy: iPSCs to treat macular degeneration (12)
	First approved stem cell-based ALS therapy by South Korea (Neuronata-R) (13)
2018	First approved stem cell-based SCI therapy by Japan (Stemirac) (14)
2019	First evidence of viable mature β cells differentiated from human ESCs (15)
2021	First stem cell therapy to treat Parkinson's disease using MSCs (16)

HSCs: haematopoietic stem cells
ESCs: embryonic stem cells
iPSCs: induced pluripotent stem cells
SCI: spinal cord injury
ALS: amyotrophic lateral sclerosis
MSCs: mesenchymal stem cells

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9552054/

Stem cell therapy (SCT) in regenerative medicine

- **Regenerative medicine** aims to treat and cure diseases by using living cells to either replace or repair diseased and damaged areas of medical interest.
- Stem cell therapy (SCT) is regarded as the frontier of regenerative medicine
- SCT is a therapeutic tool used to treat or prevent diseases through the use of human stem cells:
    1. Embryonic stem cells (ESCs)
    2. Induced pluripotent stem cells (iPSCs)
    3. Adult stem cells (ASCs)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9552054/

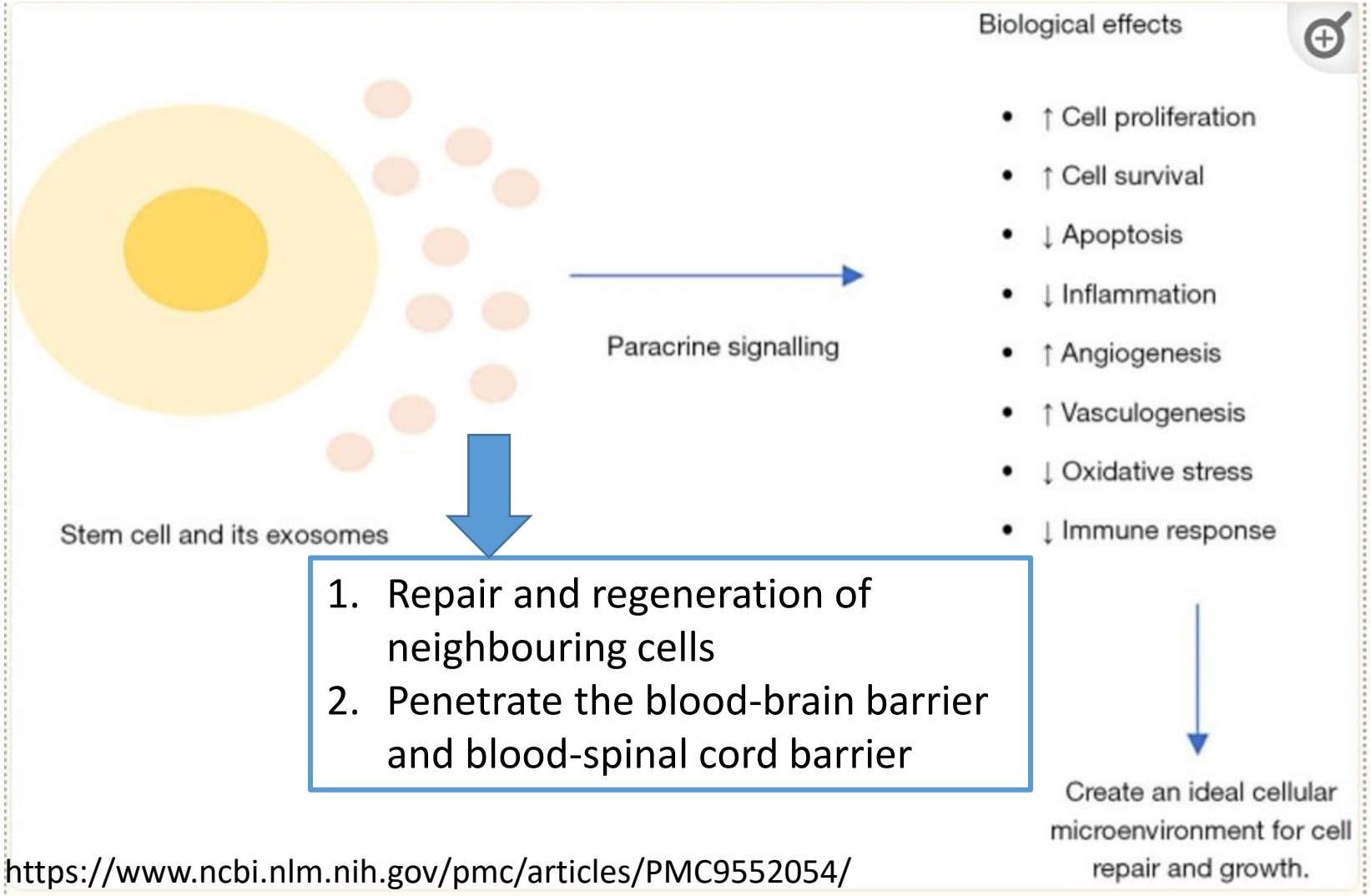
Therapeutic potential of stem cells

- Heal or regenerate damaged body tissues and congenital defects

1. ability to **self-divide into various** types of **cells**
2. releasing **paracrine factors stored in exosomes** (cytokines, chemokines, growth factors and extracellular matrix molecules catalysing the repair of injured tissues)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9552054/

Paracrine signaling of stem cells via exosomes



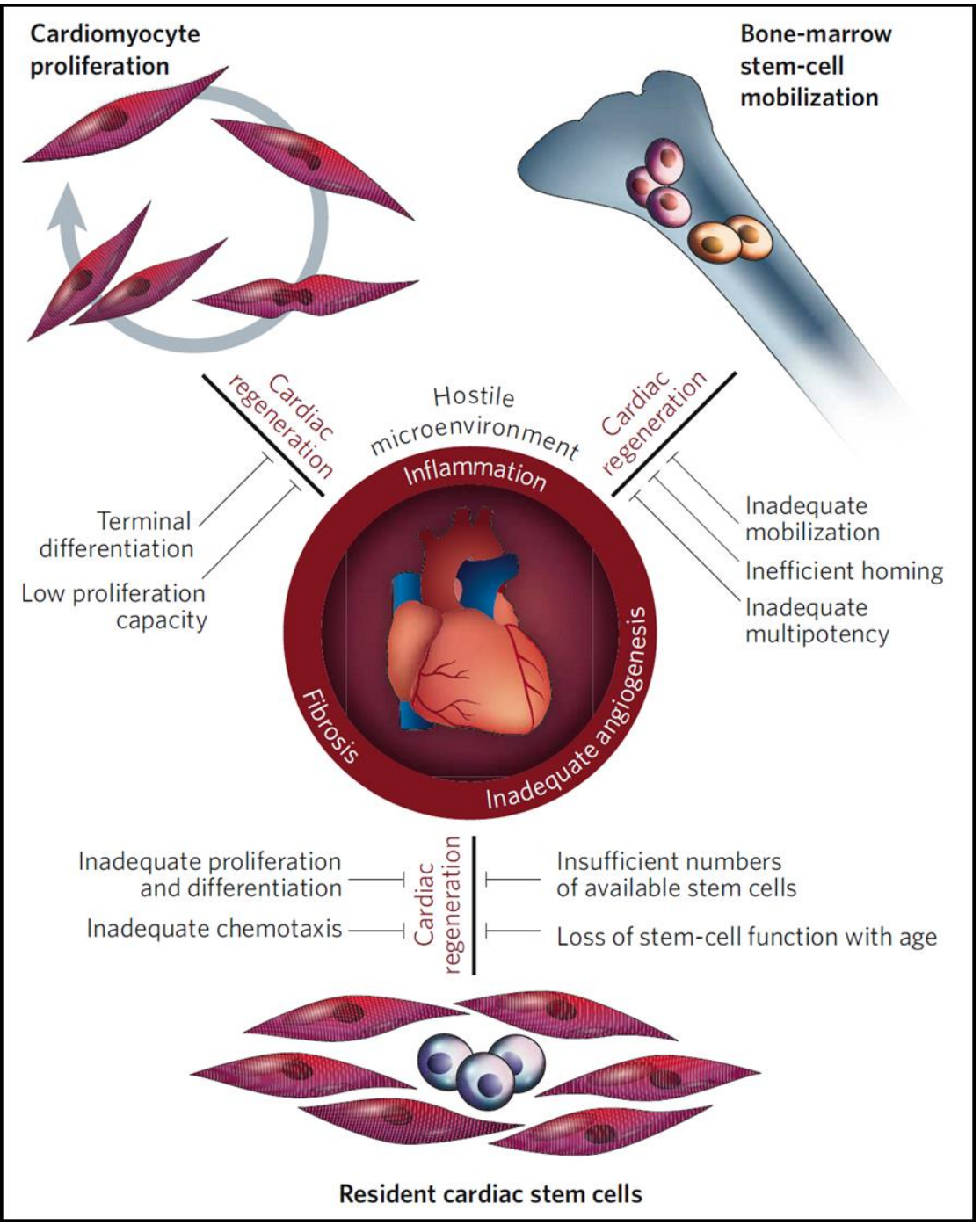
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9552054/

„**Cell-free therapy**" with the administartion of exosomes derived from SCs??

Reparative effect, without introducing living stem cells that can be potentially rejected by the body and have tumourigenic properties.

Stem-cell therapy for cardiac disease

- Heart failure is the **leading cause of death** worldwide.
- **Current therapies** only **delay progression** of the disease.
- Laboratory experiments and recent clinical trials suggest that **cell-based therapies can improve cardiac function**.
- Bone marrow-derived **progenitor cells** and other progenitor cells **can differentiate into vascular cell types**, restoring blood flow.
- More recently, **resident cardiac stem cells** have been shown to differentiate into multiple cell types present in the heart, including cardiac muscle cells, indicating that the heart is not terminally differentiated.
- These new findings have stimulated optimism that the progression of heart failure can be prevented or even reversed with cell-based therapy.



Segers & Lee, Nature, 2008

Mechanisms of, and potential barriers to, endogenous cardiac regeneration

- **Cardiomyocyte**: proliferative capacity is limited in the adult mammalian heart
- **Bone-marrow-derived cardiomyocytes:** have been detected at very low rates in adult hearts
- **Resident cardiac stem cells (CSCs)**: potential to differentiate into multiple myocardial cell types, but unknown barriers prevent endogenous CSCs from regenerating myocardium more effectively

Cell types and mechanisms proposed for cardiac therapy

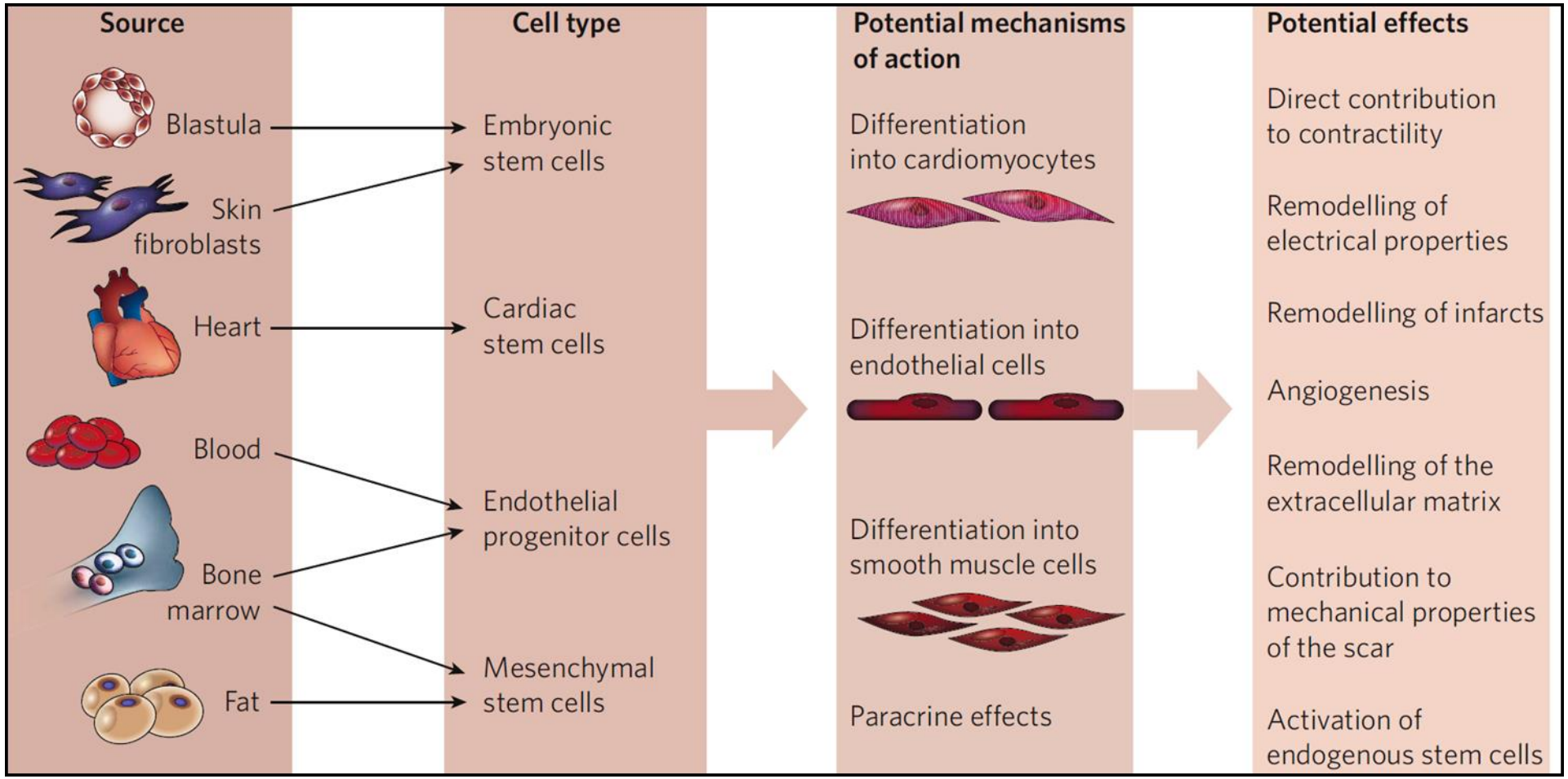


Figure 2. Many cell types and mechanisms have been proposed for cardiac therapy. **Stem cells and progenitor cells can be isolated from either autologous or allogeneic** sources. Different types of stem cell and progenitor cell have been shown to improve cardiac function through various mechanisms, including the **formation of new myocytes, endothelial cells and vascular smooth muscle cells**, as well as through **paracrine effects**.

Segers & Lee, Nature, 2008

Stem cell therapy for hematological diseases

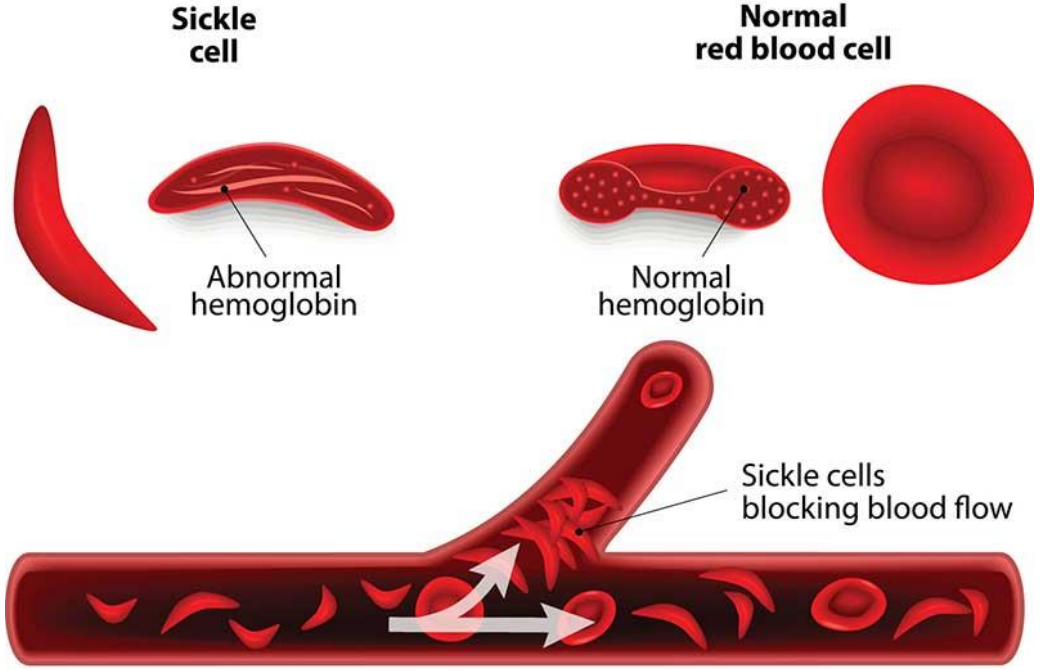
- Haematological diseases **affect the components of the blood** and the circulatory system.
- Currently, the curative therapy for haematological diseases is **autologous or allogeneic HSCT** with the aim to reconstitute and replace defective blood components
- **Disadvantages of allogeneic HSCT** :
  - High risk for GVHD
  - HSCT is dependent on donor availability

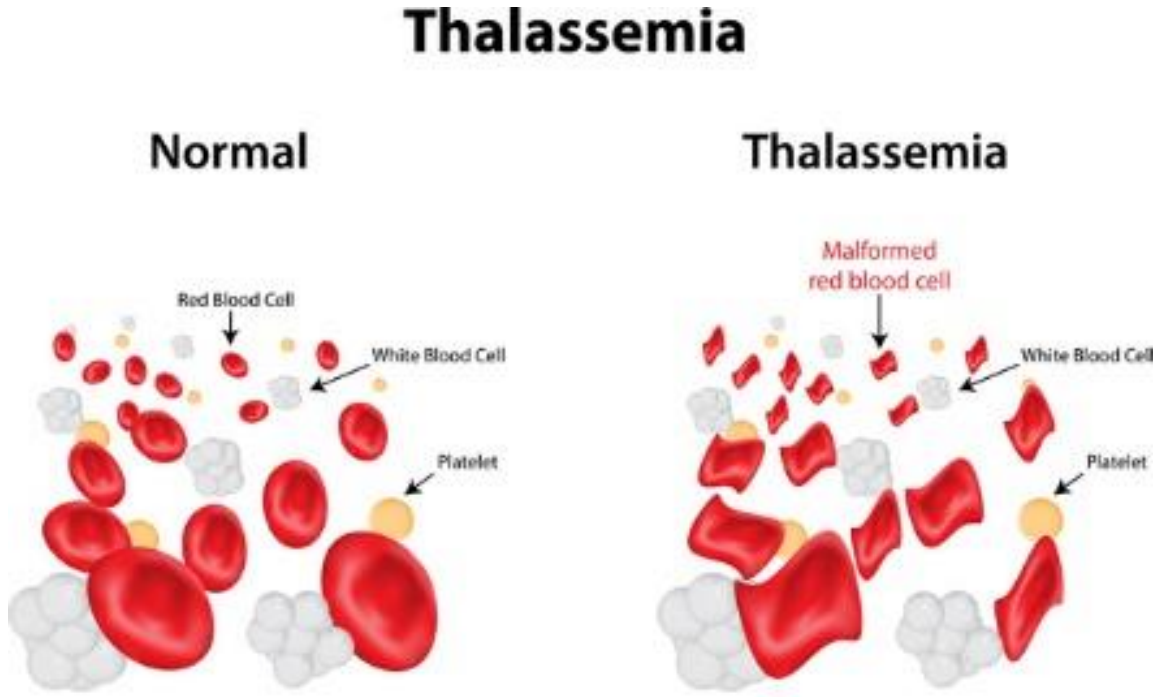
Gene-edited haematopoietic stem and progenitor cells (HSPCs)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9552054/

SCT of Sickle Cell Disease and ß-Thalassemia

https://www.froedtert.com/sickle-cell-disease/symptoms





- **Gene-edited haematopoietic stem and progenitor cells (HSPCs)** as a treatment option was investigated in patients with SCD and TBT
- **CRISPR-Cas 9 gene editing** was used to **disrupt *BCL11A* genes in HSPCs**, increasing the expression of foetal haemoglobin → **normal blood levels during follow-ups**

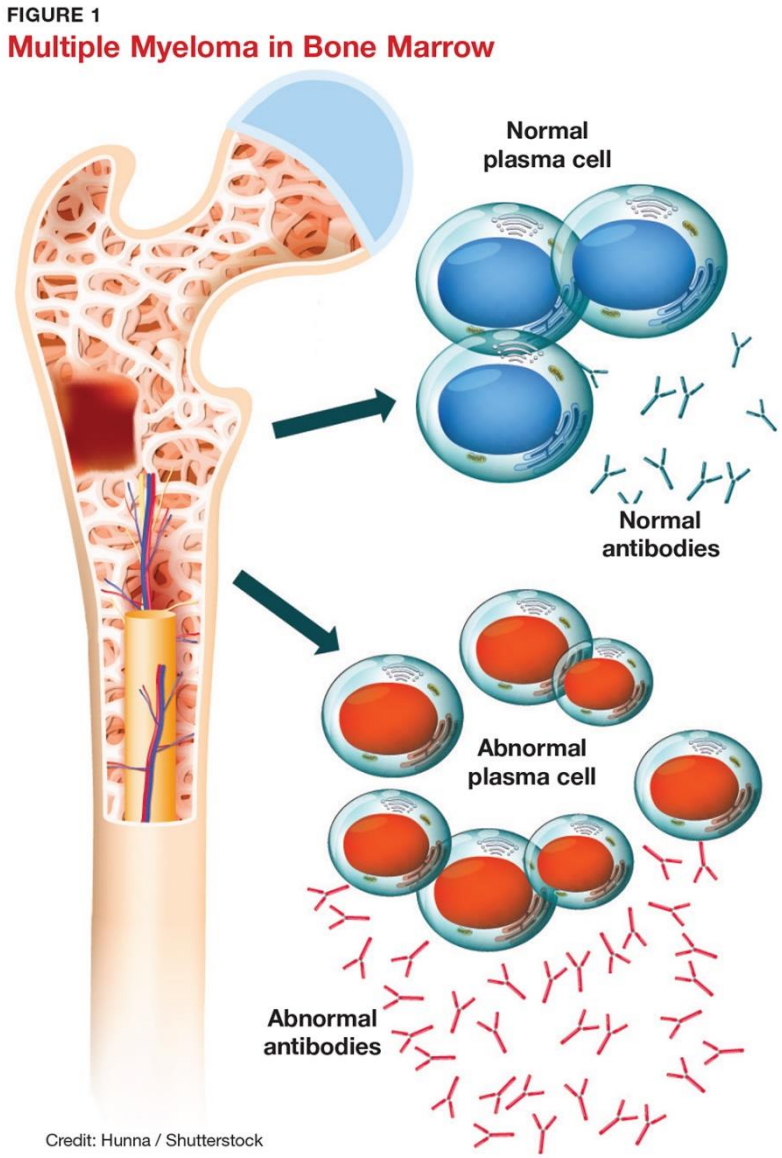
SCT for Multiple Myeloma

- MM is a **malignancy of plasma B cells**
- Abnormal proliferation of plasma cells

fatigue osteomalacia and kidney failure

- **Treatment**: autologous or allogeneic stem cell transplantation (relapses can be observed)
- **New approaches**: (Shah *et al.*) clinical study with cord blood-derived NK cells infusion along with autologous stem cell engraftment in treating 12 MM patients.

Positive outcomes, but the **efficacy** of the treatment is **indeterminable**.



Neurological diseases

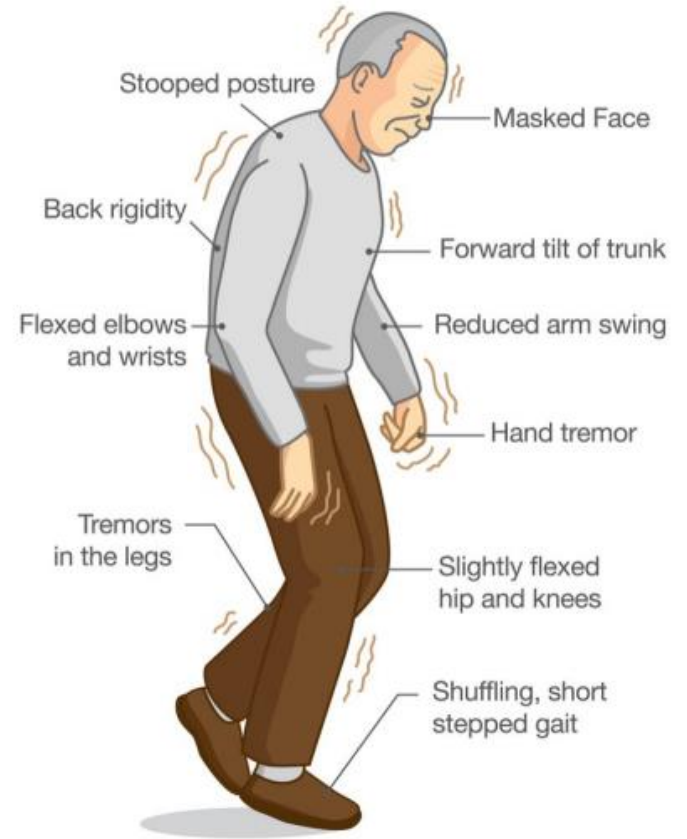
- **Increasing prevalence**
- Global Burden of Disease Study in 2016, neurological diseases have the **highest morbidity and disability-adjusted life year (DALY)**
- Many researchers are looking into **SCT as a potential treatment** for neurological diseases

Slow down the progression

Reverse the underlying disease pathogenesis

Several **clinical trials** to investigate the therapeutic effects of **Mesenchymal Stem Cells** (**MSCs**)

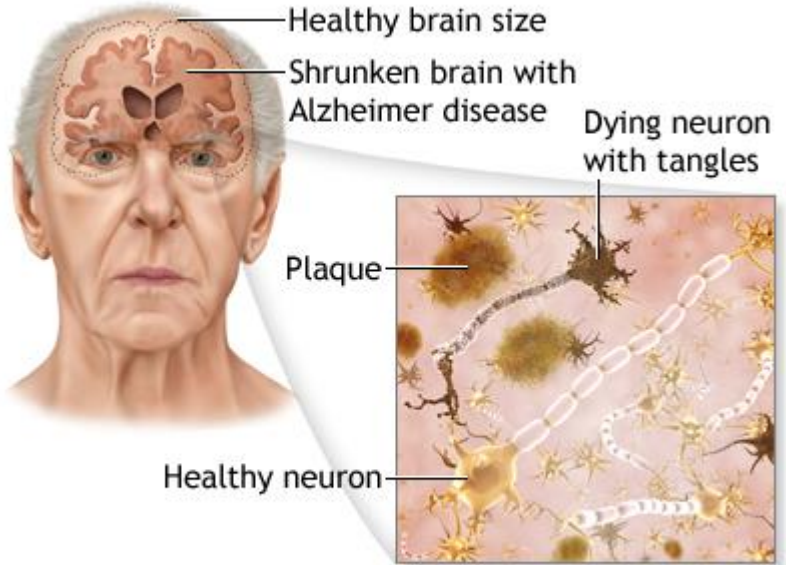
Neurological diseases to be treated



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9552054/

Alzheimer's disease:

- accumulation of **beta-amyloid plaques**
- neuronal loss and death
- decline in cognitive impairment
- only one FDA-approved drug (removal of amyloid plaques)
- transplanted MSCs with paracrine effects, exosomes



https://medlineplus.gov/ency/article/000760.htm

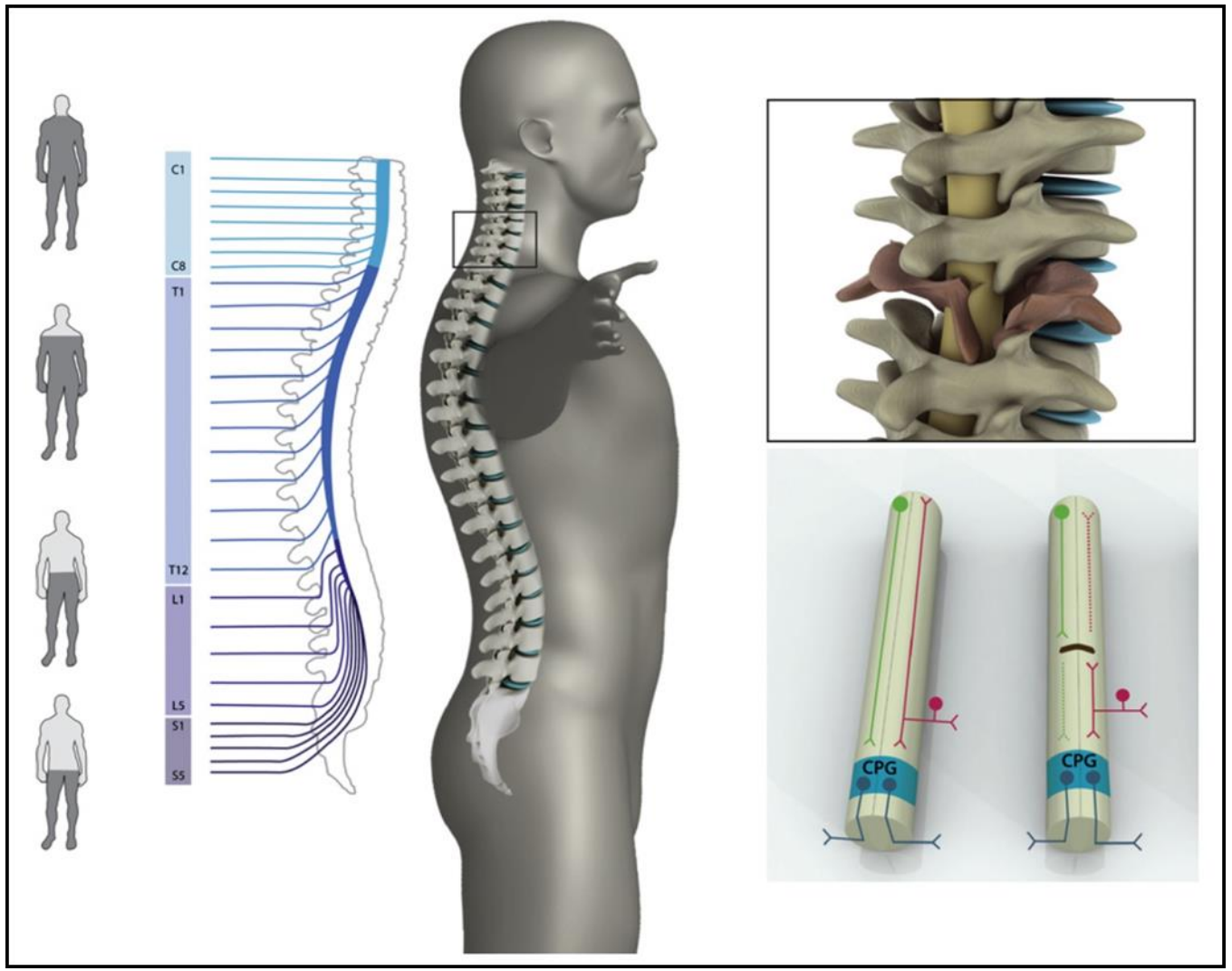
Parkinson's disease:

- affecting motor control and cognitive abilities
- rapid **loss of neurons in the substantia nigra**
- **decreased dopamine production**
- decreased efficacy of available drugs after years of usage

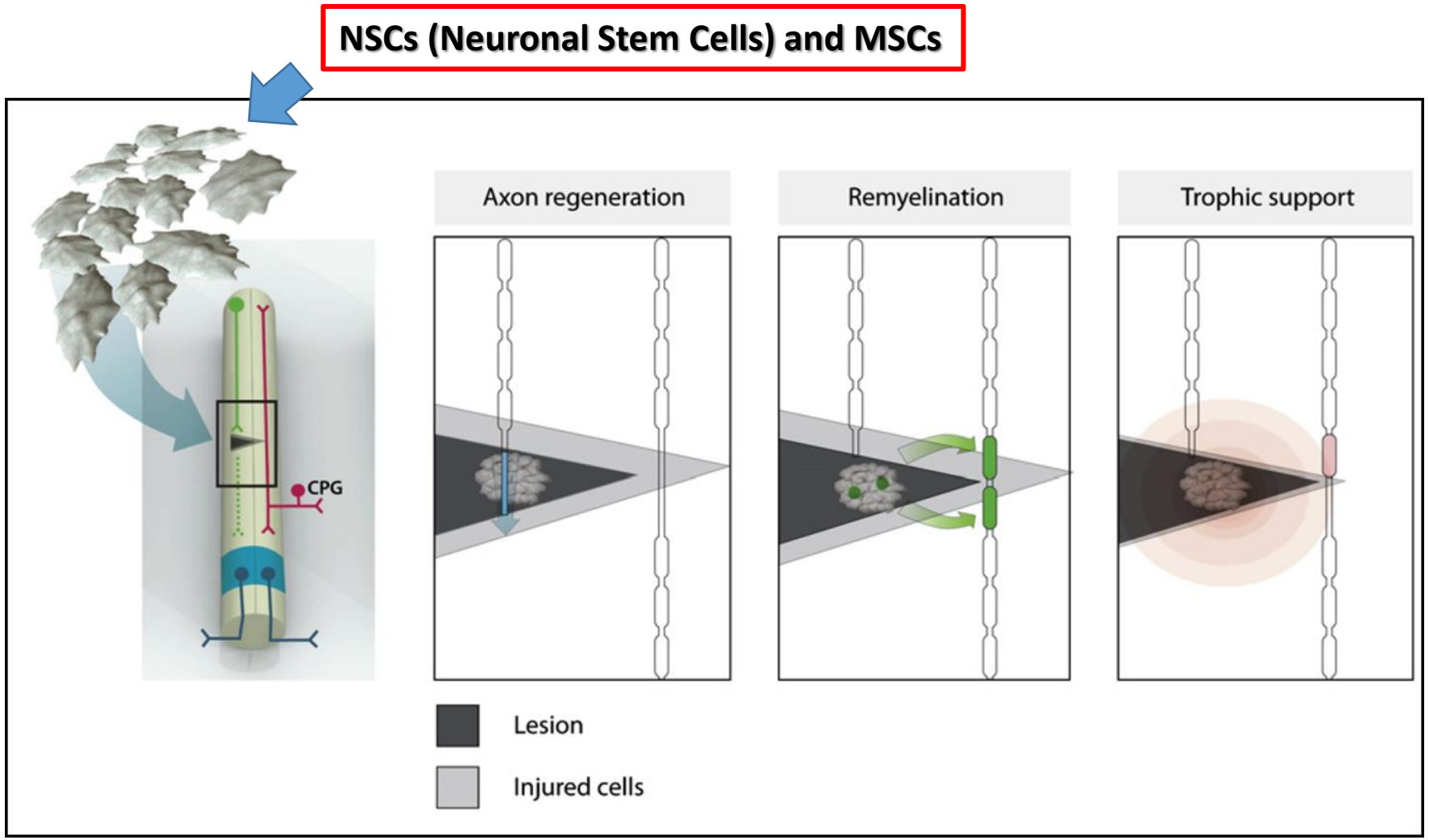
Preclinical studies with UC-MSCs, BM-MSCs, iPSCs

Stem cells for spinal cord repair

- Intractable and debilitating condition caused by a **primary injury to the spinal cord** (most often caused by motor vehicle accidents)
- **Motor control and sensory input is lost below** the level of **the lesion** (shaded area in figures to the left) due to the severance of long ascending sensory (red) and descending motor (green) axons

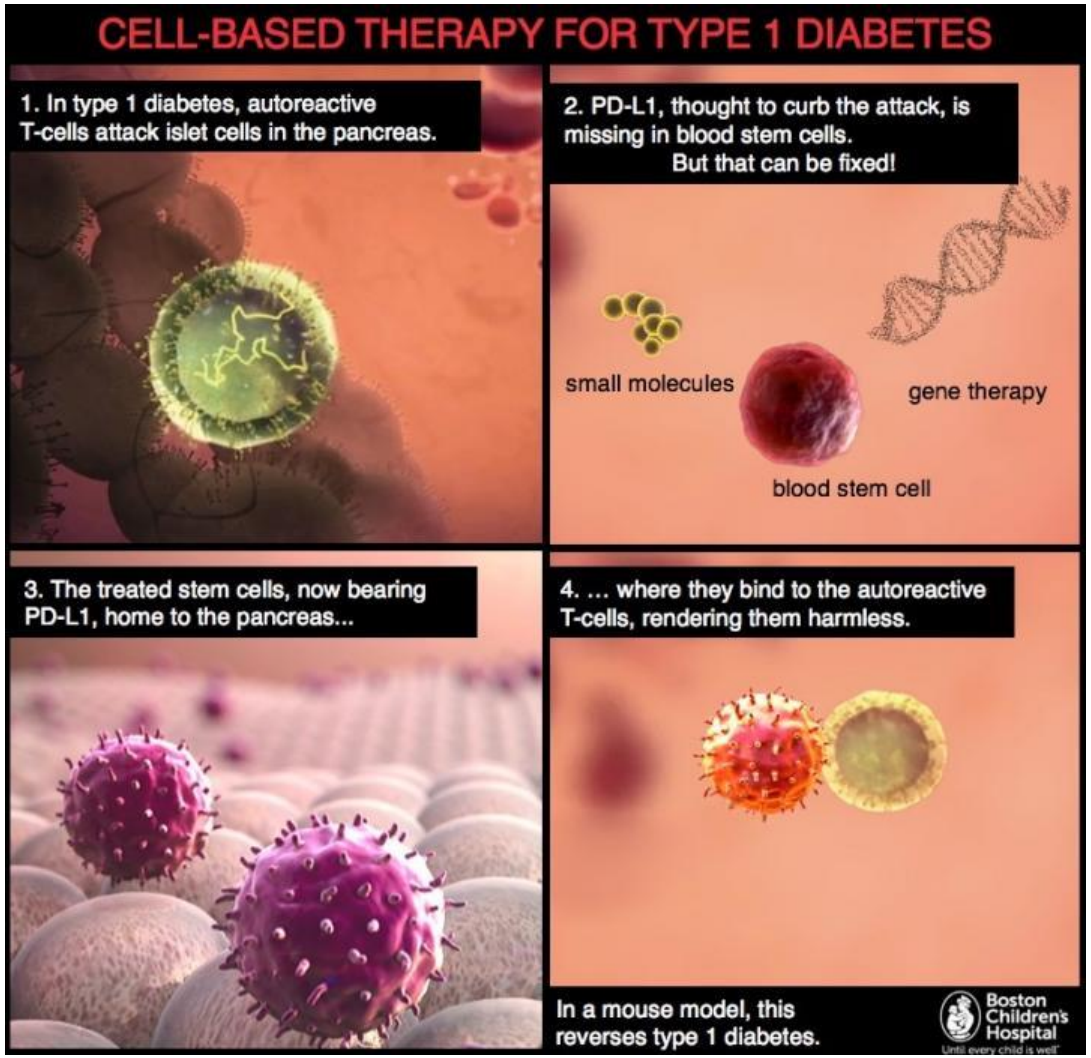


Mechanisms by which transplanted stem cell-derived cells may facilitate regeneration



- Create a permissive substrate for **axonal growth**
- Promoting **remyelination**
- Supplying **trophic support** reducing the damage
- **Rescue neurons and oligodendrocytes**
- Enhance **axonal plasticity**

Stem cell-based Therapy for Type 1 Diabetes



- Autoreactive T-cells attack insulin-producing Islet cells in the pancreas
- The Stem cells of the mice were collected
- Healthy gene for PD-L1 was delivered into the stem cells, using a viral vector
- Genetically modified Stem cells were reinfused
- The treated cells reversed diabetes in the mice

https://www.eurekalert.org/news-releases/819987

Challenges in stem cell therapy development

- Uncertainty in the underlying stem cell mechanism
- Ethical issues with ESCs
- Genetic instability (oncogenesis, leading to tumorigenesis)
- Immune rejection (usually non-autologous sources)