CNS

Biochemistry

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هذا الشيت شامل الفايل الثاني لمادة دكتورة ديالا (يعني تقريبا النص الثاني من المحاضرة الثانية)

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Stem Cells and Neurodegenerative Diseases 

Neurodegenerative Diseases: are diseases that affect the CNS in which one type or more than one type of neurons are damaged (certain tissues in CNS are damaged due to different reasons).

- A wide range of acute and chronic conditions in which neurons and glial cells in the brain and spinal cord are damaged and lost.
- **Acute**: Ischemic stroke or spinal cord injury
- **Chronic**: Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), or Alzheimer disease (AD).
- There are some considerations when using stem cells as a treatment method for one of these diseases. Must consider the disease itself, what changes occurred to induce this disease, cell type that needs to be regenerated, treatment method, and if it is worth using this treatment.

Main considerations when using stem cells to treat neurodegenerative diseases:

- **The variability between neurodegenerative diseases in the degree of disability that they cause and in the therapeutic options that are available** (e.g. Parkinson disease - symptomatic treatment →without treating the underlying cause or problem)

- For example, Parkinson disease patients can live with their disease with the available symptomatic treatments and can also use new discoveries that make their everyday life easier because their disease symptoms don't completely interfere with their daily life and it is not a life threating condition. Thus, stem cell therapy would have to provide substantial improvement (such as to return them to their normal state) to be considered as a treatment option, otherwise it is unworthy treatment for them. On the other hand, in a paralyzed patient with spinal cord injury and a high degree of disability, so here the patient has more difficult situation and his condition interferes completely with his daily life (life threating condition), so even a small percentage of improvement would be acceptable reason for the patient to undergo stem cell treatment such as improving him from a paralyzed person to one using walker. That's why stem cell treatment is measured relatively.

- **Risks to the patient that are acceptable, depending on disease severity. Animal models may not fully predict their toxicity, occurrence of immune and other biologic responses, and risk for tumor formation after implantation in patients.**

- Can stem cell transplantation cause other more severe problems? For example if undifferentiated pluripotent stem cells were transplanted to PD patient, and then they caused cancer, in this case it is better for the patient to stay in his condition with his symptoms than to have cancer! So clinicians must balance whether the stem cell treatment is worth or not.

- **The cell type that needs to be regenerated and transplanted in the following neurodegenerative disease:**

  PD – Dopaminergic neurons

  ALS – Motor Neurons

  Stroke, spinal cord injury and Alzheimer's Disease – Several cell types

- What type of cell will be used? And where will the cell be taken from? Will it be necessary to perform a major invasive operation so that the patient may be exposed to complications that are actually more dangerous than the disease he is suffering from? so in this case we can conclude that this treatment is not worth due to its risks. So always weigh the risks and benefits.

- **The stem cell-based approach should show substantial improvement of functional deficits in animal models before their use in clinical application.**

- What is the amount of improvement after using stem cell treatment? Is it a substantial improvement, or not? for example improving a paralyzed patient to one using walker is a substantial improvement, but in the other hand a PD patient, if he doesn't return to his almost his normal state, for him this is not a substantial improvement.

- **To determine the biological mechanism underlying the observed effects of a stem cell-based treatment in an animal model (e.g. reconstruction of neuronal circuity)**

- What is the biological mechanism by which stem cells restore the damage? Not just the amount of improvement must only be considered but also the mechanism of this improvement. Examples of such mechanisms:
  - Stem cells undergo differentiation into certain neuronal cell types that replace the damaged neurons.
  - Stem cells secrete soluble factors that activate the neural stem cell populations that are already present in the patient's body to be able to differentiate and replace the damaged cells.
  - The transplanted stem cells guaranteed an appropriate niche for these cells.
  - Stem cells themselves secrete molecules that restore the damage.

Note: Knowing the biological mechanism takes a lot of time (that's why mechanistic studies take a lot of time).

- Placebo effect also plays a huge role (the psychological factor); which is a beneficial effect produced by the treatment, which cannot be attributed to the properties of the placebo itself (no active substances) and must therefore be due to the patient's belief in that treatment.
  - **Double-blind placebo studies** are considered the ideal for stem cells research. In this case, both the patient and operator do not know whether the patient is receiving the actual treatment or a placebo (the control group).

- **What is required for the stem cell-based approach to be clinically competitive?**
- **The easiness to differentiate stem cells to neurons. For example, the differentiation of IPSCs into mature neurons is more complicated than for ESCs.**

We need to consider:

- If the patient needs to be immunosuppressed before the treatment begins due to suspected immune rejection
- The potential side effects (the patient must be aware of the potential side effects that may happen, and they must be less severe than the problem that is already present)
- The safety of cellular therapy administration (doesn't transmit infections such as HIV)
- Inclusion and exclusion criteria (are there certain conditions that must be present for the patient to be considered for this type of treatment).
    - Exclusion criteria (e.g. not all patients with stroke can be treated by stem cells but there are exclusion criteria such as for patients with other systemic diseases who will have higher failure rates).
    - Inclusion criteria may be certain age or degree of disease severity.
- Ensure that expectations are realistic and explained to the patient, this reminds us of the importance of doctor-patient communication, so every medical practitioner must improve his communication skills with patients such as: how to simplify information for patients because there is a lot of information that the patient doesn't need to know, and how to deliver the important information in an understandable way to all patients regardless of their educational level (from the illiterate to the one who has PhD).If you don't still know the patient level of education, then you can ask. And if you know the educational level for the patient then you can spread awareness.

Inclusion/exclusion criteria	Enrolling late-stage patients may prevent loss of quality of life Late-stage patients may mask any positive effects due to the intervention occurring too late in the disease course
Realistic expectation	Informed consent forms must clearly illuminate the goals of the study Safety trials vs. efficacy trials Expectations of therapeutic effects based on disease state at intervention
Controlled study	Ideal study is a double-blind placebo study Late-stage patients may mask any positive effects not observed due to the intervention occurring too late in disease Original PD studies offered control arms treatment after a 1-year follow-up which confuses interpretation of efficacy
Immunosuppression	While the brain remains an immunologically privileged site due to the blood-brain-barrier, there is evidence that this barrier can be compromised in disease Studies into cell graft survival demonstrate that immunosuppression increases that survival of graft tissue
Potential side effects	Prevent/minimize potential side effects (i.e. meningitis, fever) Avoid exacerbation of disease and tumor formation Risk vs. quality of life
Safety of cellular therapy administration	Consider CNS accessibility and safety of delivery methods Pros/cons of systemic delivery, lumbar puncture or stereotactic injection are important

Examples on Neurodegenerative Diseases Targeted by Stem Cell therapy:

Parkinson's Disease (PD)

The problem with Parkinson disease is in the dopaminergic neurons, in which these neurons are damaged or their number is less than normal. In PD the damage is in **just one cell type** which makes the problem relatively simpler, so the problem is solved by replacing this cell type OR by replacing its product which is dopamine, that's why PD patients are given L-Dopa (remember L-Dopa give rise to dopamine, norepinephrine, and epinephrine in its pathway)

Characteristic symptoms are rigidity, hypokinesia, tremor, and postural instability

Degeneration of nigrostriatal DA neurons is the main pathology

Tx: l-DOPA, DA agonists, enzyme inhibitors, and deep brain stimulation

No Tx for dementia

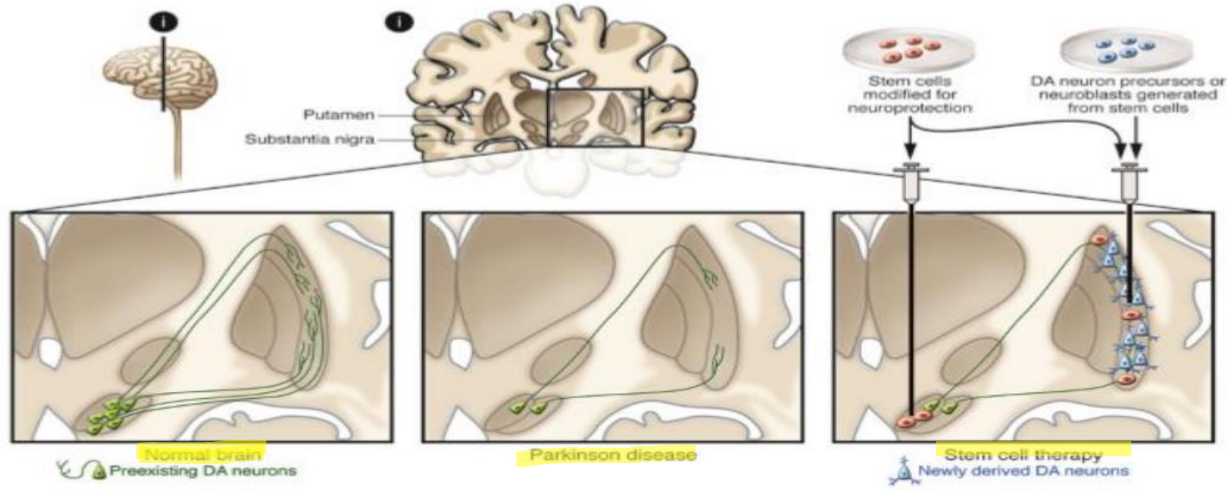
iPSCs for modelling the genetically complex PD

- We can use stem cells in PD in 2 ways: Either to transplant the cells that have been differentiated to dopaminergic neurons, or to transplant the stem cells that can be induced by the surrounding to get differentiated into dopaminergic neurons.

Stem Cell-Based Therapies for PD:

Don't memorize details.

Proof of principle: clinical trials with intrastriatal transplantation of human embryonic mesencephalic tissue (rich in postmitotic DA neuroblasts).



More dopaminergic neurons and more synapses in normal brain.	In PD: number of cells are less with less synapses, which will cause the problem. Note: in PD there are some dopaminergic neurons but less than normal.	When using stem cell-based therapy for PD, they tried transplanting different types of cells of different sources AND found that they were able to generate dopaminergic neurons and able to secrete dopamine, so the damage is restored.

- **These experiments are still under research and have their pros and cons:**

Pros	Cons
The DA neurons that formed from the transplanted tissue were able to secrete dopamine and reinnervate the denervated striatum and become functionally integrated into the tissue (make synapses just like normal neurons). They were able to restore striatal DA release and gave rise to clear symptomatic relief in some patients.	Even there is no disease progression, but small fraction of graft-derived DA neurons contained Lewy bodies (characteristic and hallmark for PD). *Availability of human embryonic mesencephalic tissue is limited (the tissue from which the cells they used were derived from), which means no enough source of cells to be transplanted.
11-16 years after transplantation, cell replacement remained a viable therapy (the cells lasted for a long time and are still active, they knew this by labelling the transplanted cells and thus they are distinguished from the cells that are already present)	There was high variability of functional outcome after transplantation (there is high variability in response to treatment, so there is no specific predictable outcome, sometimes the response will be 40% and in another patient it may be 60-70%. So the range is highly variable and unpredictable).
The progression of pathology in graft-derived neurons is slow (which means that disease severity does not increase after transplanting), and they are still functional after a decade.	Poor standardization of the transplanted cell material contributes to high variability (there is no clear step by step process to follow in order to prepare such treatment this will lead to unpredictable range of results which make stem cell therapy more difficult to apply).

Other sources of DA Neurons (all are still under study)

- ✓ ES cells
- ✓ Cloned ES cells
- ✓ NSCs and progenitors of embryonic ventral mesencephalon
- ✓ Adult NSCs from the subventricular zone (SVZ)
- ✓ Bone marrow stem cells
- ✓ Fibroblast-derived iPS cells

Human stem cell–derived DA neuron precursors/neuroblasts can survive in animal models of PD and can be functional after maturation.

Hurdles that prevent stem cell therapy for PD to jump from bench (laboratory) to clinic:

- **PD is a multisystem disorder. If nondopaminergic systems are affected, they will not improve by intrastriatal DA grafts** (Parkinson is a multisystem disorder that involves several systems and its pathological changes are highly variable between patients, so it will be difficult to guarantee results).
- **Substantial re-innervation of striatum has not been demonstrated** (transplanted cells must be able to re-innervate and to reform connections with surrounding cells).
- **Restoration of DA release in vivo has not been demonstrated** (must guarantee that the transplanted dopaminergic neurons are able to secrete dopamine)
- **Marked improvement (50-70%) in the deficits and symptoms experienced by PD patients has not been demonstrated** (Parkinson is not a devastating disease, so there must be a great amount of improvement to consider stem cells a treatment option)
- **Risk of tumor formation**, even if minor, is not acceptable.
- **The need to inject cells at all sites of injury** (which may be practically difficult).

Clinical Trials

Do not memorize. | The doctor just read what is highlighted.

There are a lot of ongoing experiments now, but none of them have reached the point to be approved as a treatment option for PD.

By International Stem Cell Corporation (ISCO)

- Parthemogenetic cells derived of unfertilized oocytes after suppression of the second meiotic division.
- Drawbacks: Used cells are PAX6-positive suggesting that they are of a dorsal neural fate. In contrast authentic midbrain dopaminergic neurons are derived from a PAX6- negative ventral midbrain neural precursor. (PAX6-positive is a transcription factor that induces some negative effects)

• Schwarz et al. used human-fetus-derived dopaminergic neurons and transplanted them into PD patients' depleted striata.

• Takagi et al. demonstrated functional recovery after successfully transplanting dopaminergic neurons from monkey ESCs into PD patients' brains.

• Another study also confirmed the influential role of undifferentiated ESCs in functional recovery by differentiating dopaminergic neurons in a PD rat model

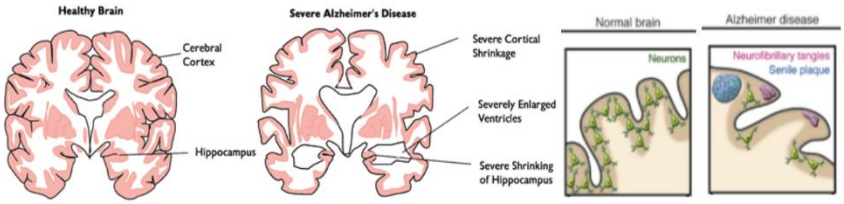
• Two other clinical studies using human ESCs are ongoing in Australia and China, where their preclinical studies' results were reported

• Garitaonandia et al. described the preclinical tumorigenicity and biodistribution safety in vitro before conducting a phase I clinical trial to evaluate the safety and tolerability of stem cells for the treatment of PD

Alzheimer's Disease (AD)

Alzheimer's disease →accumulation of beta amyloid→ that will cause destruction of: **multiple types of neurons**, supporting cells, and their ECM. Which means that there is regional tissue damage. That's why stem cell therapy for AD is more **difficult** to use, as we need to:

1- replace a tissue rather than replacement of one cell type
2- Reinnervate the synapses.

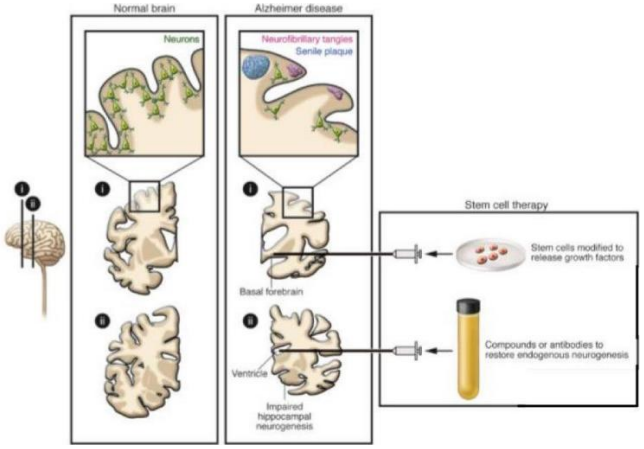


Memory impairment, cognitive decline, and dementia due to widespread and progressive pathological changes

Neuronal and synaptic loss, neurofibrillary tangles, and deposits of β-amyloid protein involve the basal forebrain cholinergic system, amygdala, hippocampus, and cortical areas.

Stem cell–based therapies for AD:

Many experiments have been tried whether in stem-cell based therapy or other treatments. Examples:



1- **Cholinergic neurons: Acetylcholinesterase inhibitors**→ which enhance cholinergic function because acetylcholinesterase inhibitors inhibit the destruction of acetylcholine so it will remain functioning→ this will induce some temporary improvement in AD patients (not long-lasting improvement).
2- **Neurogenesis or maturation of hippocampal neurons** as the formation of immature hippocampal neurons was reported in AD (induced in the hippocampus).
3- **Nerve growth factor (NGF) releasing stem cells** to stimulate regeneration and repair of neurons (release of this growth factor induces changes in the tissue that improve the situation).
4- **Anti-β-amyloid antibodies** or β-amyloid-degrading protease neprilysin (destruction of beta amyloid aggregates may solve the problem).

Hurdles that prevent stem cell therapy for AD to jump from bench (laboratory) to clinic:

- Stem cells have to be pre-differentiated in vitro to many different types of neuroblasts for subsequent implantation in many brain areas (Remember multiple neuron types have to be replaced in AD, which means tissue replacement).
- Synapses must be replaced and reinnervated.
- For a long-lasting symptomatic benefit, cholinergic cell replacement and differentiation requires intact target cells in the transplantation site (host neurons that the new cholinergic neurons can act on) that are damaged in AD.
- Stem cell-based replacement strategies are very far from clinical application in AD.

Clinical Trials (very primitive)

Do not memorize.

The doctor just read what is highlighted.

•By stemedica cell technologies

• Stem cells from healthy people to mild to moderate AD patients

• To test if stem cells work for AD

• The clinical trials for AD treatment are still in their infancy, where the FDA approved the first clinical trial of MSCs(using mesenchymal stem cells) for AD treatment in 2015 (still there is no results).

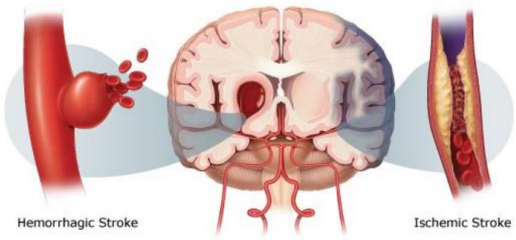
• Similar trials are currently underway or under development in Europe and Asia to assess the safety, tolerability, and preliminary efficacy of human MSCs in patients with mild-to-moderate AD

• Recently, combining stem cells with NGF was recognized as a useful strategy for preventing cell death, stimulating the growth of cholinergic neurons, and facilitating the generation of specific neural populations in AD treatment.

Stroke

Stroke is defined as occlusion of a blood vessel in the brain which will decrease the blood flow to the area supplied by it. Some blood vessels are more susceptible to be occluded than others, the most common artery to be occluded is the middle cerebral artery. Generally the presentation and complications of stroke depend on many factors including which artery has been occluded, the **location** in that artery, and the **duration** for the occlusion.

Ischemic stroke, caused by occlusion of a cerebral artery, leads to focal death of multiple neuron types, as well as oligodendrocytes, astrocytes, and endothelial cells.



Neuronal plasticity and reorganization of neural circuitries contribute to spontaneous recovery to varying degrees, but most patients exhibit persistent motor, sensory, or cognitive impairments

- What makes the usage of stem cells difficult in cases of stroke is that in stroke we **lose a tissue rather than one cell type which is similar to Alzheimer**. So according to the types of cells in

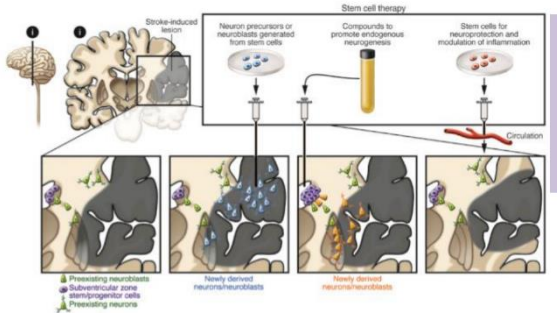
this tissue which depends on which region is affected, certain stem cells may be used and given time to re-innervate the synapses and establish their communication.

- Many patients with stroke will exhibit improvement in their symptoms after **physical rehabilitation**, in this case their symptoms will become less severe and their functionality will improve this is due to the idea that there are neuronal stem cells that are already present in human body that help in this process.

- Another factor that helps the brain adapt to stroke and improves the patient's symptoms is that the area surrounding the infarct (infarcted/hypoxic region) will gradually increase the number of **peroxisomes** which are specialized in reducing oxidative stress and regeneration. This process takes time.

Stem cell–based therapies for stroke

Human ES cell–derived NSCs and MSCs, grafted into rat stroke site, migrated toward the lesion and improve forelimb performance.



IV injection of human NSCs induced improvements after hemorrhagic stroke in rats, probably through antiinflammatory actions

Stem cell–based therapies for stroke

- ✓ No substantial clinical improvements were detected after IV injection of autologous MSCs in patients with an ischemic lesion in the regions supplied by the middle cerebral artery (MCA).
- ✓ Several clinical studies using intravenous or intraarterial (into damaged territory) infusion of autologous bone marrow–derived stem cells in stroke patients are ongoing.
- ✓ A clinical trial in stroke patients involving transplantation of clonal, conditionally immortalized NSCs isolated from human fetal cortex is being tested.
- ✓ 80% of neuroblasts and neurons die during the first two weeks after formation at stroke site in rats.

The doctor just said this:

*What makes the usage of stem cells **difficult** in cases of stroke is that in stroke we lose a **tissue** rather than one cell type which is like Alzheimer.

Clinical trials:

- Transplanted ESCs, iPSCs, and NSCs can replace the missing brain cells in the infarcted area
- Non-neuronal adult stem cells, such as MSCs provide trophic support to enhance self-repair systems such as endogenous neurogenesis

The doctor just said this:

*Even though stem cell therapy is difficult to use in stroke, but there are clinical trials that used different types of stem cells (embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and neural stem cells (NSCs))

*These trials are still under study

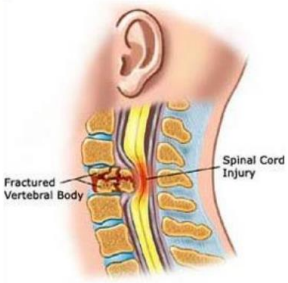
Spinal Cord Injuries:

The usage of stem cells is also **difficult** and complicated in cases of spinal cord injuries because a tissue with different cell types is lost rather than one cell type, additionally there is a need for reinnervation, and reconnection of the synapses that are damaged.

Pathological changes after spinal cord injury are complex and include:

1. Interruption of ascending and descending pathways
2. Loss of neurons and glial cells
3. Inflammation
4. Scar formation
5. Demyelination

- ✓ Patients experience loss of movement, sensation, and autonomic control below the level of the injured spinal segment.
- ✓ Available treatments are ineffective.
- ✓ Different types of stem cells were tested and improved functional outcome in animal models through **secretion of neurotrophic factors, remyelination of spared axons, or modulation of inflammation**



Conclusion: Stem cell therapy or any other new treatment is a long process that takes a lot of time (at least 15 years) and needs so much patience, starting from lab experiments on cellular level, to small animal models to large animal models to clinical trials (with its all stages), and finally testing the treatment on the long run.

We should not be intimidated by any external factors throughout this process.

The lecture has ended here. Next are the rest of the slides.

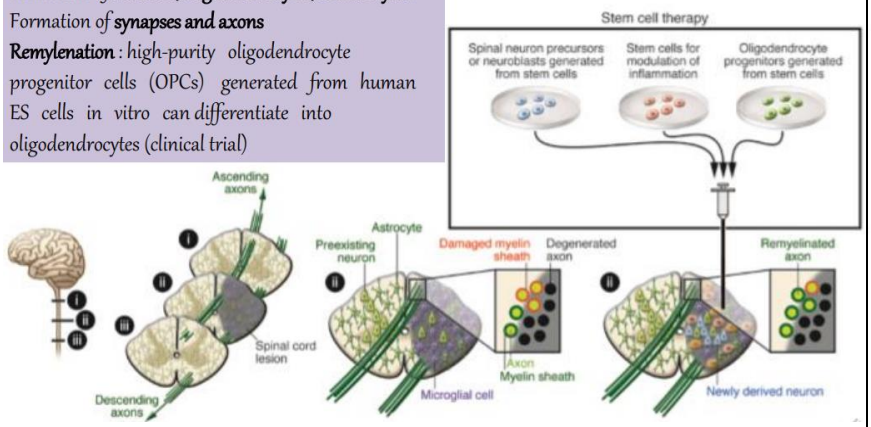
Stem Cell-Based Therapies for Spinal Cord Injuries:

Before moving to clinic:

Determine how to control the proliferation of transplanted stem cells and their progeny.

Determine how to enhance the differentiation of these cells to the specific types of neurons that have been lost.

Formation of **neurons, oligodendrocytes, & astrocytes**.
Formation of **synapses and axons**
Remylenation: high-purity oligodendrocyte progenitor cells (OPCs) generated from human ES cells in vitro can differentiate into oligodendrocytes (clinical trial)



Determine how the resulting neurons can be directed to format appropriate synaptic contacts.

Other stem cell types: Umbilical cord blood, bone marrow–derived HSCs, and MSCs have already been applied in patients with spinal cord injury, with claims of partial recovery.

Problems in these trials:

1. The implanted cells were often poorly characterized.

2. The preclinical evidence of efficacy for several of these approaches was insufficient.

3. The therapeutic benefit was reported from open-label trials where patients had been subjected to physiotherapy.

4. The mechanisms underlying observed improvements were unclear.

Hindrances in stem cell therapies' progression:

1. Learning how stem cells work in the body and how they integrate with the targeted tissue/organ.

2. The safe and cost-effective generation of these cells in adequate quantities. For example, ESCs and iPSCs can be grown indefinitely in the lab, but the procedures are very complex and demanding, limiting these cells' overall availability.

3. Risk of post-implant rejection, which adds the burden of needing a close compatible donor for the cells' recipient.

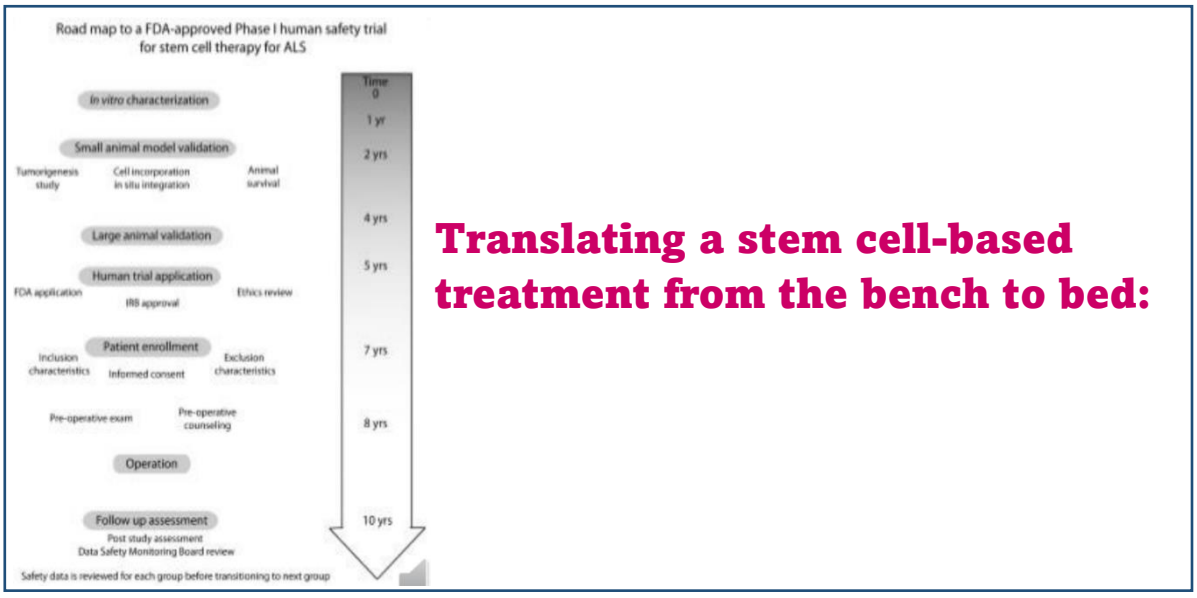
4. Identifying the proper conditions to culture these cells, the most suitable route of administration, delivery, and the target site

5. Most of the data available to researchers were derived from animal studies, hence, direct extrapolation of the results from these in vivo studies into human patients is not feasible at this stage.

6. The cost, time, and labor-intensive nature of stem cell therapy limit its use, especially in developing countries. Additionally, safety considerations, such as the potential for malignant transformation and side effects, such as epilepsy, immune allergic reactions, and injection site injuries, remain significant concerns.

Neurodegenerative Diseases & Stem Cell Therapy:

- Clinical trials using stem cells have already been performed or initiated (e.g., for the rare, fatal, autosomal recessive neurodegenerative disorder Batten disease)
- No stem cell–based therapy has yet been proven beneficial for any neurodegenerative condition.
- Despite this fact, unproven treatments for several neurodegenerative diseases are offered at "clinics" around the world without rationale and with poor scientific and clinical basis.
- Ethical, regulatory, societal, and economical issues need to be addressed.



Translating a stem cell-based treatment from the bench to bed:

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بالتوفيق يا رب