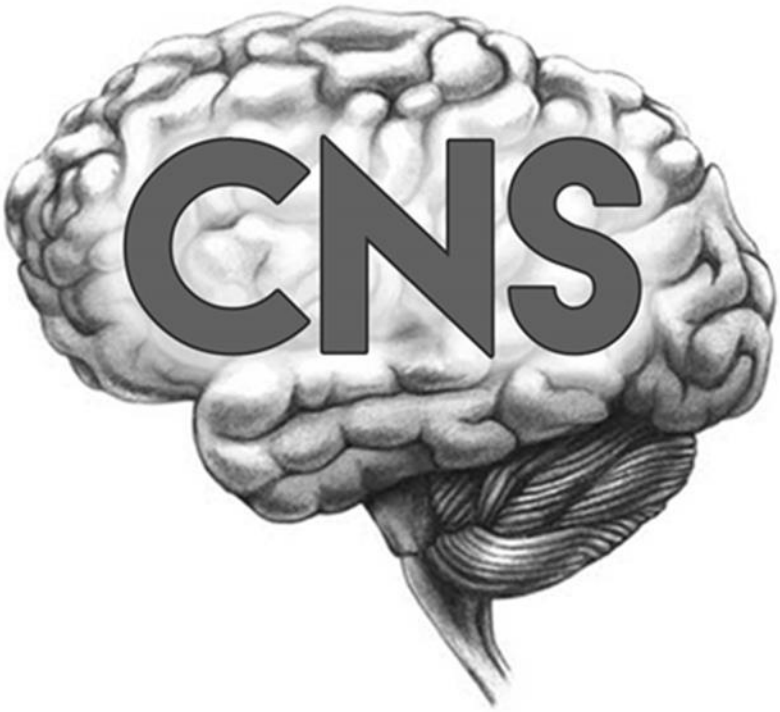

Biochemistry

Sheet ○ Slide ○ Handout

Number

4

Subject

Stem Cells and Neurodegenerative Diseases

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Doctor

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Date:

Price:

**** This sheet was written according to the recording that belongs to section 3. Please pay attention that <u>the order of ideas in this sheet is a little bit different from that in the recording</u>.**

****Almost all the content of the slides of the lecture is included within this sheet.**

*****What has been written in italic was not mentioned by the doctor, but is mentioned in the slides.***

❖ Before we start,

This sheet is concerned with reviewing the progress achieved in the context of "Stem Cell-Based Therapy" for treatment of some common diseases of the CNS.

According to Dr. Diala, **most of this lecture is NOT for memorization**. <u>The things you will be asked about in the exam are limited to the following:</u>

- The take-home messages (in the last page)
- The steps that any stem cell-based therapy has to pass in order for it to move from a "research topic" to an "approved treatment". (this is very important ethically).
- When it comes to the diseases discussed in this sheet, you should only memorize the information related to the stem cell therapy. For example, for each disease:
  - what are the things lost (specific type of neurons, multiple types, myelination,…)?
  - what are we trying to correct (or replace) using stem cells in this disease?
  - What makes stem cell therapy in certain disease (Parkinson's for example) more encouraging than in another disease (like Alzheimer's)?
- Stem cell biology (the topic of the previous lecture (sheet 3))

In the exam, <u>you will NOT be asked about the following:</u>

- The trials that were carried out for certain diseases and if they succeeded or not.
- Details about the diseases (like symptoms, pathogenesis) <u>IF THEY ARE **NOT** RELATED TO THE STEM CELL-BASED THERAPY TOPIC.</u>

❖ Topics of this lecture:

*Introduction.

*Main considerations when we use stem cells in the treatment of neurodegenerative diseases.

*Steps of translating a stem cell-based treatment from bench to bed.

*Stem Cell-Based therapy in Parkinson's disease, Alzheimer's, Strokes, and Spinal cord injuries.

*Important take-home messages.

❖ Introduction

Neurodegenerative diseases are a wide range of acute and chronic conditions in which neurons and glial cells in the brain and spinal cord are lost.

- *Acute: ischemic stroke or spinal cord injury*
- *Chronic: Parkinson's Disease (PD), Alzheimer's Disease (AD), Amyotrophic lateral sclerosis (ALS)*

In this lecture, we will focus on the use of stem cell therapy for Parkinson's Disease (PD), Alzheimer's, Ischemic strokes, and spinal cord injuries. All of these conditions share a common feature that is "Damage of Neurons". So how can we restore the function of the damaged parts of the brain using stem cells?

It's important to mention that stem cell-based therapy for some neural diseases has reached the stage of clinical trials, yet **it has NOT been approved** for treatment of any of them.

Many issues stand in the way of the approval. The most important of these are the safety issues and the ability to restore the normal function (the efficacy).

Four important questions should never leave your mind when we're talking about the use of stem cells in this field:

- Is it -in reality- **approved** to be used in the clinic?
- Is it **ethical**?
- Is it **safe**?
- Is it **efficient**?

The most important "take-home message" from this lecture is: Don't believe the propagandas (دعايات أو أخبار مُبالغ فيها) you hear every now and then from the media. For example: when you read titles like "A research group discovered a treatment for disease X using stem cells", keep in your mind that you have to be very careful before applying this treatment in your clinic. Even if a paper proved that using stem cells for treatment of disease X in animal models was successful, it is unethical to transfer the technology used to your clinic for the purpose of having something special in your clinic/ center/ career/ experience…

It is possible to succeed with some of your patients, but don't forget that you might hurt others and cause their condition to get worse.

Notes from the slides:

- *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.*

❖ The Main considerations when we use stem cells in treatment of neuro-degenerative diseases

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

  When we talk about neurodegenerative diseases, the stem cell-based therapy should be able to improve the life of the patient by a large degree. For example, why would a patient with Parkinson's disease -who could just take his L-DOPA and other medicines- decide to try the expensive Stem cell-based therapy that might be associated with dangerous side effects?

  →**Treatment by stem cells must be able to improve the life of the patient by a large degree to be considered an encouraging option for treatment**.

- *Animal models may not fully predict their toxicity, occurrence of immune and other biologic responses, and risk for tumor formation after implantation in patients.*

  If a treatment succeeds in animal models -even if in higher animals- it does not have to be successful in human beings.

  "We're NOT big mice"

  -Dr. Issa Abu-Dayyeh

  Moreover, we should not forget that one of the problems associated with stem cell use -especially when we're talking about pluripotent stem cells- is the possibility of cancer formation (carcinogenesis).

- *The variability between neurodegenerative diseases in the degree of disability that they cause and in the therapeutic options that are available.*

  The disease of interest might have a specific type of neurons affected or it may affect many types. As a result, we need to replace "the damaged part" whether it's
  - a specific type of neurons *(as in the cases of **Parkinson's** and **ALS**, in which the target is to replace a specific type of neurons → dopaminergic neurons, and motor neurons, respectively)*
  - or several types of cells (as in the cases of strokes and Alzheimer's in which the target is to replace the damaged "tissue")

- The cell type to be regenerated and transplanted.
  - PD: dopamine neurons
  - ALS: motor neurons
  - Stroke and Alzheimer's disease: several cell types

- ***Risks** to the patient that are acceptable, depending on the **severity**.*

- *To determine the biological mechanism underlying the observed effects of a stem cell–based treatment in an animal model. E.g. reconstruction of neuronal circuitry.*
  Once we decide to use stem cells as a treatment, we have to know what is the mechanism of action.
  {knowing that "It works" is not enough. We should know the mechanism and how the effect is achieved. Examples:
  - Did the replacement itself fix the condition? (in other words, are the transplanted cells functioning instead of the lost cells?)
  - Are the transplanted cells stimulating the neighboring cells to divide and repair the tissue?
  - Are the transplanted cells secreting certain molecules that stimulate the internal stem cells inside the tissue to differentiate?}

  → Knowing the mechanism of action is very important. For example, let's assume that it turned out that the transplanted cells secrete certain molecules responsible for the therapeutic effect, it might be successful to inject only those "certain molecules" instead of transplanting the whole cells.

❖ Translating a stem cell-based treatment from bench to bed

The approval of any treatment -including stem cell-based therapy- has to pass through many stages that include:

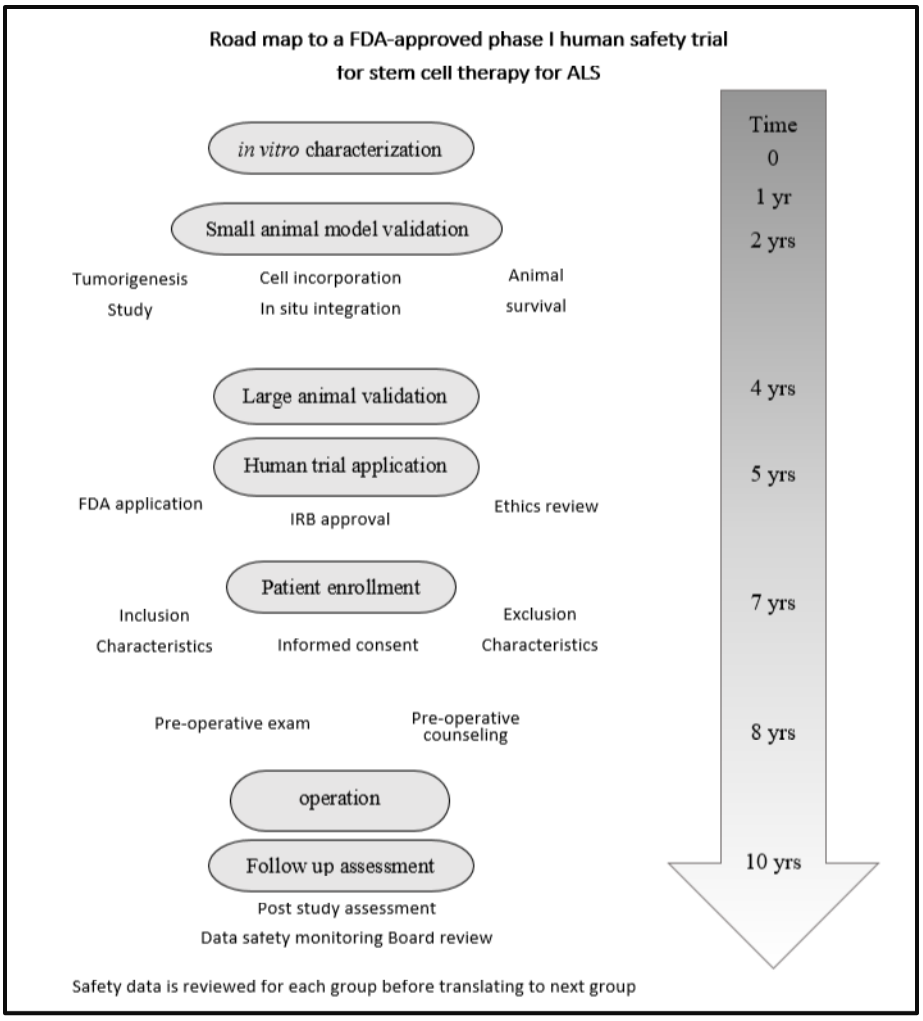
- Cell cultures or organ cultures (**in vitro** experiments).
- Followed by in vivo experiments using **small animals**.
- Then moving to **higher (large) animals**; like monkeys, pigs…etc.
- After passing the previous stages, we can move on to **clinical trials**.
  - Clinical trials include several stages (phases). It's important to mention that using the method under investigation on your patient (as a treatment) is not allowed in the first phases of the clinical trials.
  - The first phase is usually concerned with checking the safety of the treatment (safe/toxic). Other phases include checking the required dose and frequency of usage, in addition to many other important steps.

Note: some patients -especially those in the end stages of their disease- become hopeless and ready to try anything they hear about in the media.

(المريض يتعلَّق بقشّة)

It is your responsibility to inform the patient and let him know if he's going to be part of a clinical trial, or part of an approved treatment.

- We should **assess the treated subjects on the long run** (we should check for possible long-term side effects/ complications that might develop in the patients as a result of the treatment).



Notes:

- The only FDA-approved treatment using stem cells is Bone Marrow Transplantation. It took 60 years from the "idea" to finally reaching the clinic as a safe treatment method. This does not necessarily mean that we will need 60 years for the approval of any stem cell-based therapy. However, it will at least take approximately 15-20 years. (The advanced technology nowadays might make the process faster).

- Dr. Diala was the first to try transplanting stem cells in a certain tissue in the eye called "The Trabecular Meshwork" (she worked on cadaver eyes). If this method will reach the clinic one day, it will need 15-20 years because there are many steps that need to be passed, and many questions that need to be answered. Examples:
  - Is it **safe?**
  - What will be the source of the cells?
  - How to differentiate them?
  - What is the dose and what is the frequency?
  - What is the appropriate mode of transmission (e.g. injection)?
  - What is the media through which the cells will be transferred to the site of injury? (for example, we might need the cells to be in an aqueous media with specific characteristics).
  - And many other questions.

- Common considerations when translating stem cell therapies to neurodegenerative disease patients include:
  - Are there going to be any complications?
    (you have to tell your patient about the possible complications and risks, both; in written and oral forms)
  - Do I need to suppress the immunity of the patient or not?
    (this depends on the source of the transplanted cells. Are they autologous -from the patient himself- or from another person?)
  - Other points are illustrated in the table: (memorize the main points on the left, and just read the rest).

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

Abbreviations: PD, Parkinson's disease; CNS, central nervous system.

❖ Parkinson's Disease (PD)

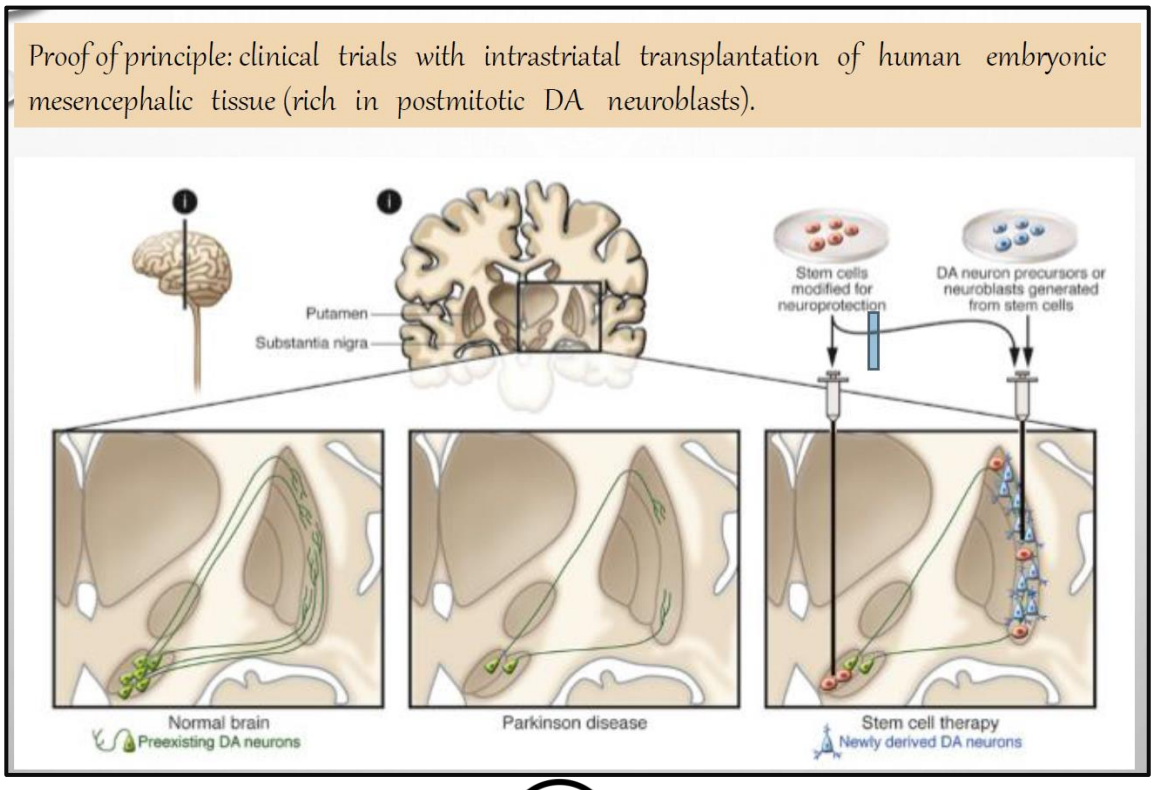
I. About the disease

- The problem is in the dopaminergic neurons in substantia nigra (degeneration of nigrostriatal DA neurons is the main pathology).
- *Characteristic symptoms are: rigidity, hypokinesia, tremor, and postural instability.*
- The traditional treatment:
  - L-Dopa (to compensate for the decreased dopamine), dopamine (DA) agonists, some enzyme inhibitors, *or deep brain stimulation.*
  - *There is no treatment for dementia.*

II. Stem cell -Based therapy for PD

Look at the figure below and pay attention to the following notes:

- The left part of the figure shows the normal DA neurons of substantia nigra with their axons projecting in large numbers to the putamen.
- The part in the middle illustrates how the number of DA neurons is markedly decreased in Parkinson's disease.
- In the trial of stem cell therapy (the part to the right), they transplanted a special type of stem cells derived from human embryonic mesencephalic tissue at certain sites.
  The cells were monitored over time, things were okay, and newly derived DA neurons were formed in different regions of the brain.



III. Advantages and disadvantages (Pros and Cons)

- The most important advantages were:
  - The used stem cells were able to settle and attach to the tissue and produce dopamine.
  - They also stayed viable for a long period of time (up to years).

- The most important disadvantages were:
  - The availability of these cells (human embryonic mesencephalic tissue) is limited.
  - The variability of functional outcome after transplantation is high among different patients.
  - Poor standardization of the transplanted cell material (the receiving patients did not get very similar types of the transplanted cells in terms of the source and the preparation method). This contributes to the high variability.
- *Take a look at the following table (from the slides)*

Pros	Cons
-The DA neurons that form from the transplanted tissue reinnervate the denervated striatum and become functionally integrated, restoring striatal DA release and giving rise to clear symptomatic relief in some patients.	-A small fraction of graft-derived DA neurons contain Lewy bodies (the hallmark of PD). - Availability of human embryonic mesencephalic tissue is limited.
11–16 years after transplantation, cell replacement remains a viable therapy.	Variability of functional outcome after transplantation is high.
The progression of pathology in graft-derived neurons is slow, and they are still functional after a decade.	Poor standardization of the transplanted cell material contributes to the high variability

IV. Other types of stem cells that were also used in trials:

- Embryonic stem (ES) cells
- Cloned Embryonic stem cells
- Induced pluripotent stem cells (Fibroblast-derived iPSCs)
- Bone marrow stem cells
- Neuronal stem cells (NSCs) and progenitors of embryonic ventral mesencephalon
- *Adult NSCs from subventricular zone (SVZ)*

Note (from the slides): Human stem cell–derived DA neuron precursors/neuroblasts can survive in animal models of PD and can be functional after maturation.

V. Hurdles (العوائق) that prevent stem cell therapy for PD from bench to clinic

(1) PD is a multisystem disorder
- Different regions in the brain might be affected → so we might need to inject different sites and that's not practical. Moreover, we might need to repeat the procedure (several injections). This is an example where the dose and frequency are key points in the treatment.
- *Since it's a multisystem disease, if nondopaminergic systems are affected, they will not improve by intrastriatal DA grafts.*

(2) *Substantial re-innervation of the striatum has not been demonstrated.*

(3) Restoration of DA release in vivo (in animal models) did not show promising results. (in vitro, the cells were able to secrete dopamine, but in vivo, the restoration of DA release has not been demonstrated).
- in vivo results are more important than in vitro results.

(4) *Marked improvement (50-70%) in the deficits and symptoms experienced by PD patients has not been demonstrated.*

(5) There's a risk of tumor formation, and even if it's minor, it is not accepted.

(6) The need to inject cells at all sites of injury *(the same as in point (1))*

Notes:

- The risk of tumor formation depends on two main factors:
  1. The type of stem cells used → if you use pluripotent stem cells like ESCs and iPSCs, the risk of tumor formation will be much higher compared to the risk that accompanies the use of mesenchymal stem cells that usually do not result in cancer formation.
  2. The state in which the stem cells are used → for example, if you use pluripotent stem cells in a differentiated stage (differentiate them, then inject them), the risk of tumor formation will be low, because the differentiated cells don't have an unlimited ability to proliferate like pluripotent stem cells.

- Stem cell-based bone marrow transplantation is considered safe because the adult stem cells used (mesenchymal or hematopoietic) do not have the ability to form cancer as embryonic or pluripotent stem cells.

VI. Clinical Trials

- One of the latest clinical trials is the one held by the International Stem Cell Corporation (ISCO).
  - In this trial, the cells used are parthenogenetic cells (cells derived from unfertilized oocytes after suppression of the second meiotic division – in other words, these cells are isolated after undergoing only the first meiotic division, so they are still diploid (2n))
  - The most important drawback is that the dopaminergic neurons come originally from PAX 6 -negative cells (PAX 6 is a transcription factor), while the cells used in this trial are PAX 6 positive.
  - This trial is still ongoing and the results haven't been revealed yet.

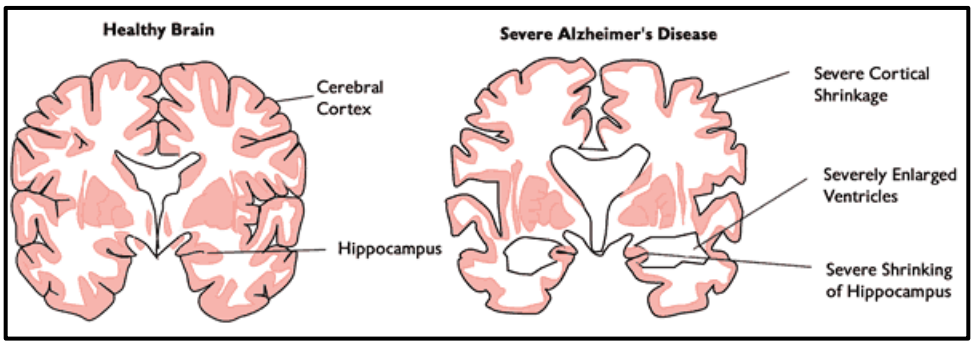
❖ Alzheimer's Disease (AD)

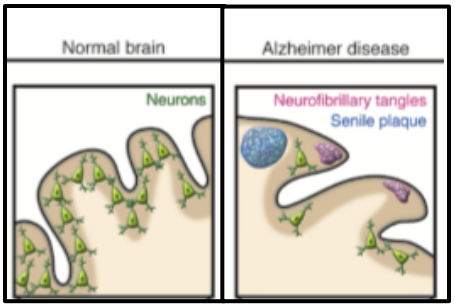
I. About Alzheimer's

- The disease involves neuronal and synaptic loss, neurofibrillary tangles, and deposits of β-amyloid protein *involving the basal forebrain, cholinergic system, amygdala, hippocampus, and cortical areas*.
- *Symptoms include: memory impairment, cognitive decline, and dementia due to widespread and progressive pathological conditions.*

Note: remembering processes and establishing knowledge in our brains depend on the formation of synapses.

- Some cases of AD are familial. In these cases, the disease starts earlier compared to other cases (approximately, when the patient is in his 40's)
- Note mentioned by the doctor: Alzheimer's is different from dementia that results from the process of "aging". In the later, we don't see the structural changes seen in Alzheimer's.
- Treatment methods for AD -whether using stem cells or other techniques- have different targets (this note will be clear after you read the treatment section).





II. Possible therapy methods for AD

(1) Trials started targeting cholinergic neurons by using Acetylcholine-esterase inhibitors.

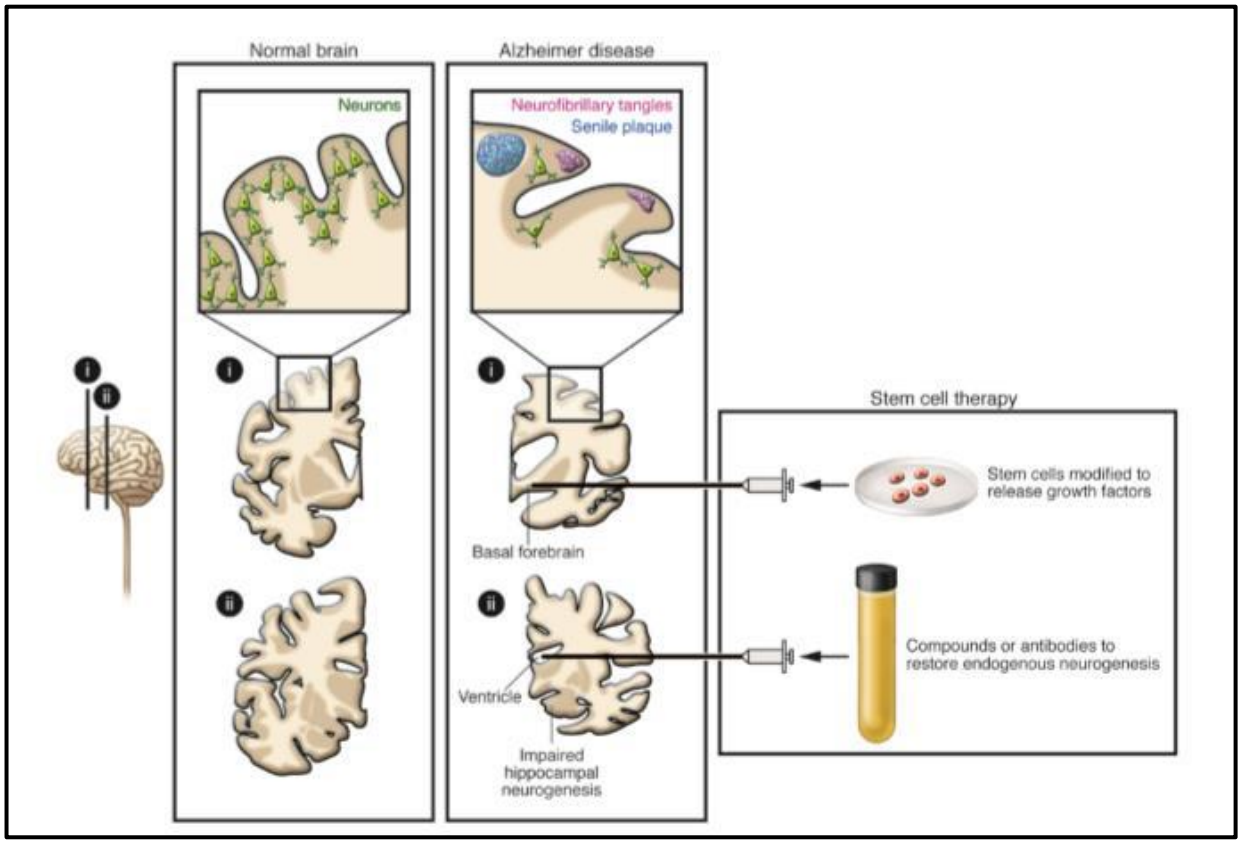
➔ If we inhibit acetylcholine-esterase, acetylcholine degradation will decrease and thus, transmission and synapses will be more efficient.

(in other words, the cholinergic function will be enhanced and some temporary improvement in AD patients will be induced)

(2) Using certain types of **stem cells** that stimulate neurogenesis or maturation of hippocampal neurons was reported in AD.

(3) It is also possible to transplant **stem cells** that release Nerve Growth Factor (NGF) to stimulate the tissue in which they were transplanted to regenerate and form more neurons and more synapses.

(4) The use of β-amyloid antibodies or certain proteases like β-amyloid degrading protease neprilysin is also possible. (to degrade the accumulated β-amyloid).



III. Hurdles that prevent stem cell therapy for AD from bench to clinic

- The situation in Alzheimer's is more complex than Parkinson's disease because in Alzheimer's there's loss of different types of cells, and thus, if we are going to treat using stem cells, we'll have to replace all the lost types of neurons (the situation is closer to replacing a "tissue" rather than replacing a specific type of neurons).

  →*Stem cells have to be pre-differentiated in vitro to many different types of neuroblasts for subsequent implantation in many brain areas.*

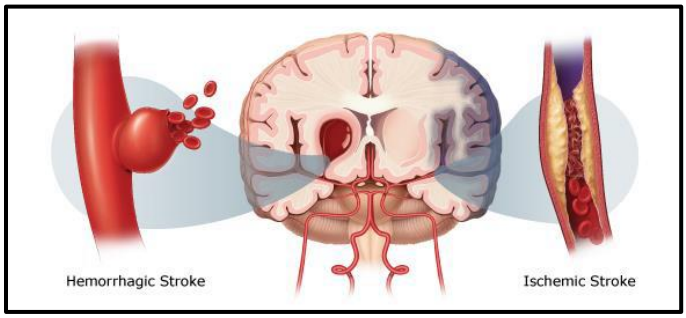
- For a long-lasting symptomatic benefit, cholinergic cell replacement requires intact target cells *(host neurons that the new cholinergic neurons can act on)*
  →The location of injection must be a healthy region and that's hard to determine using the available examination techniques.
- Stem cell-based cell replacement strategies are very far from clinical application in AD. However, some clinical trials are being carried out nowadays.

IV. Clinical trials

One of the latest clinical trials is the one carried out by "Stemedica Cell Technologies".
This company is trying to use different types of stem cells *(from healthy people to mild to moderate AD patients)* just to prove the concept *(to test if stem cells work for AD).*

❖ Strokes

I. About strokes



- The most common neuro-degenerative disease.
- Strokes involve neuronal cell death due to ischemic or hemorrhagic cell injury (here we focus on ischemic strokes).
- *Neuronal plasticity and reorganization of neural circuitries contribute to spontaneous recovery to varying degrees, but most patients exhibit persistent motor, sensory, or cognitive impairments*

Note: some of the stroke patients might improve with physical therapy. One of the factors which contribute to the restorative processes that occur is the role of peroxisomes in reducing oxidative stress.

A quick reminder (from last year's cell biology course):

In strokes, the biogenesis (formation) of peroxisomes increases in the normal healthy cells surrounding the infarct. Why?

Because the peroxisomes, via their content of anti-oxidant enzymes, reduce the oxidative stress.

→This might explain why we see some improvement in stroke patients after a period of physical therapy.

Pathogenesis of ischemic strokes:

→Ischemia leads to hypoxia

→high degree of oxidative stress

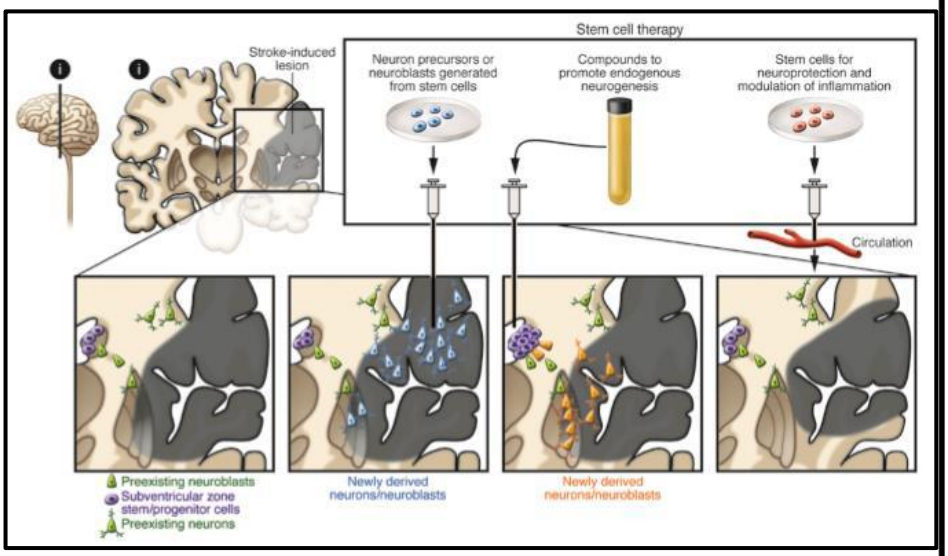
→death of neurons in the region supplied by the affected blood vessel.

(A small region will be affected if only the terminal part of a blood vessel was occluded. On the other hand, if the occlusion was in the beginning of a major blood vessel, the size of the infarct will be much larger).

- *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.*
  - ➔ So the major challenge we face when we consider stem cell-based therapy is that we have an infarct affecting a certain region with all its content.

II. Stem cell-Based therapy for strokes

- when scientists injected neural stem cells, they noticed that these cells start to attach to the tissue and start forming some synapses.
- Other types used in trials were ES cells and mesenchymal stem cells.



Notes from the slides:

- *Human ES cell–derived NSCs and MSCs, grafted into rat stroke site, migrated towards the lesion and improved forelimb performance.*
- *IV injection of human NSCs induced improvements after hemorrhagic stroke in rats, probably through anti-inflammatory actions.*

III. Hurdles that prevent stem cell therapy for strokes from bench to clinic

- 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).*
  For example, in clinical trials related to heart ischemic injuries, there's more than 50 reports proving the efficiency of stem cell therapy for treatment, thus, it's considered efficient in the cases of heart ischemia. On the other hand, there aren't many reports supporting stem cell therapy in the cases of strokes → stem cell therapy is NOT considered an effective treatment when it comes to strokes- at least, for now.
  (**<u>several</u>** groups in several areas worldwide should report the efficiency of stem cell therapy in strokes before considering it as an encouraging option for treatment)

Notes from the slides:

- *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.*

IV. 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 proved some effect in the restoration of the function of the region affected by strokes. *(these cells provide trophic support to enhance self-repair systems).*
- *There are Several types of MSCs.*
- **It is too early to conclude whether MSC therapy can improve functional outcomes in patients with stroke (too early to decide if it's effective or not).**

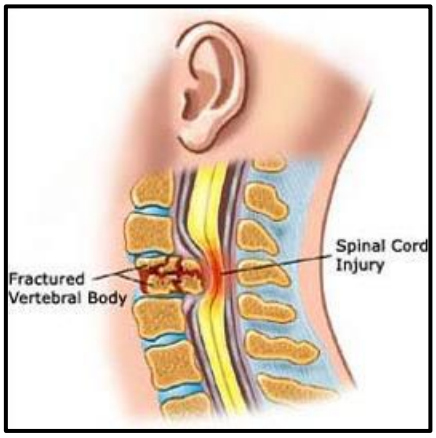
Note

When you're carrying out experiments on animal models, you have to choose your animal model carefully (it should be the closest to humans in terms of anatomy and physiology of the topic under investigation).

For example, if you want to study a specific protein that is found in humans but not in rats, it's not logical to pick the rat as your animal model.

❖ Spinal cord injuries

I. About spinal cord injuries



- Spinal cord injuries are very common, especially in road traffic accidents (RTAs) that usually involve young adults.
- The clinical outcome depends on the site (level) of injury, and the affected neurons.
- Pathological changes after spinal cord injury are complex and include:
    1. Interruption of ascending and descending pathways
    2. Loss of neurons, synapses, 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.*

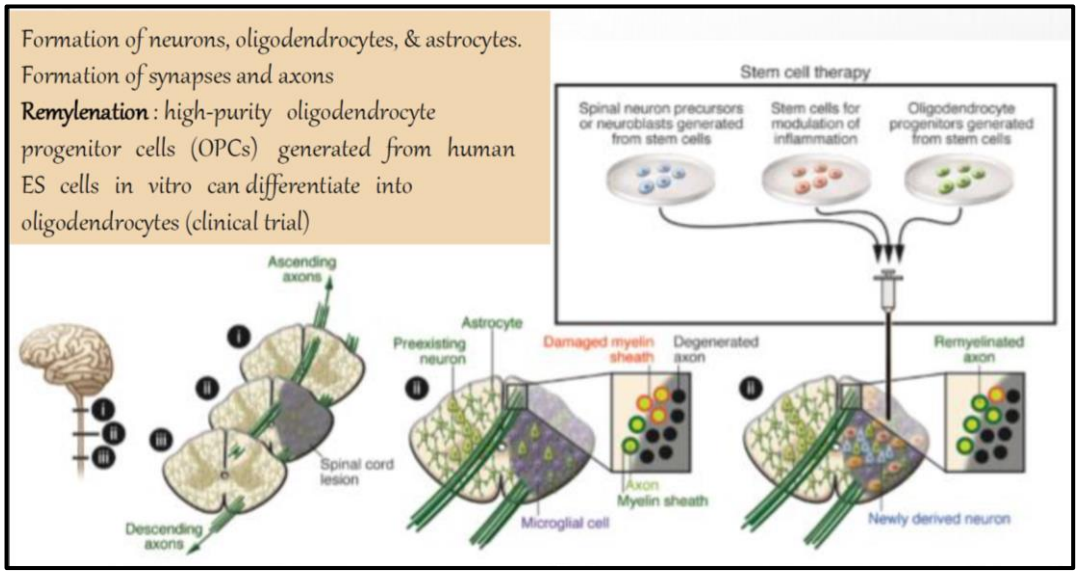
- *Available treatments are ineffective.*

- *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*

- Many factors contribute to the complexity of using stem cell-based therapy in spinal cord injuries. Of these
    - We have to replace the lost neurons, re-establish synapses, re-establish (or activate) myelination, and stop or inhibit scar formation.
    - There's a high degree of variability between patients with spinal cord injuries in terms of clinical picture, age, life style, response to treatment and many other factors.

II. Stem cell-based therapy for spinal cord injuries

- Because we need to restore many things (neurons, synapses, myelination,...), we have to use a combination of different types of cells and molecules.



- The results are promising. However, none of the trials got through to the clinic.

III. Before moving to the clinic, we need to

(1) Determine <u>how to control the proliferation</u> of transplanted stem cells and their progeny, especially if we want to use embryonic stem cells or iPSCs.
(2) Determine <u>how to enhance the differentiation</u> of these cells to the specific types of neurons that have been lost.
(3) Determine <u>how the resulting neurons can be directed to format appropriate synaptic contacts,</u> induce remyelination, and inhibit/stop scar formation or degrade it by certain mechanisms.

IV. Other stem cell types that were tried in spinal cord injuries

(1) Umbilical cord blood
(2) Bone marrow–derived HSCs
(3) MSCs
➔ *These three types have already been applied in patients with spinal cord injury, with claims of partial recovery.*

V. 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**.

❖ Important Take-home Messages

I. Don't believe everything you hear :)

II. Be safe doctors.

- Whatever your specialty in the future, you have to be very careful before transmitting any new technique to your clinic.
- Always remember the following four words: Safe, Ethical, Efficient, Approved.
- To regret NOT doing something is better than regretting doing something that might hurt your patients.

III. Before preceding to the clinic, any new treatment (whether stem cell-based or drug-based) must pass many steps {cell/organ culture, small animal models, large animal models, clinical trials…. (refer to page 5)}

For example, if a pharmaceutical company one day asks you to try a new drug that passed the first stages (in vitro), but did not undergo the next required steps, you should NOT accept trying this drug on your patients even if in vitro experiments showed promising results.

IV. You should be part of the awareness programs related to stem cell-based therapy.

For example, it is your responsibility to make it clear for patients that not everything they hear in the media about stem cell-based therapy is considered to be an "approved" treatment.

V. Finally, always remember that no one knows everything :)

I apologize for any mistake I may have made.

Wish you all best of luck :D