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# Introduction

Sometimes we forget about things, like our toothbrush when we go on a trip or calling a friend for their birthday. This is normal, it happens to us during our life and as we get older this happens more often. More than 500,000 elderly people die each year because of Alzheimer's. If this disease was cured, more than half of a million lives would be saved in a year. Between the years 2000 and 2008 deaths caused by Alzheimer's rose to 66%, meaning that it kills more than prostate cancer and breast cancer combined.[1] [2] This disease may be a joke to some, because they see it far away because of their age or because they are not aware of it.

I need to set the scene so you can understand my motivation to undertake this investigation project: Today in developing countries, the average lifespan is in the low eighties for women and the mid-seventies for men. As you move along the path to old age, you should be able to do things on your own but Alzheimer's doesn't allow, more than 25 million people that are afflicted worldwide, to do so. The motivation to do this project may sound somewhat bizarre or peculiar. During my year abroad I signed up to do volunteering in a nearby nursing home. I thought that I should, in some way, give back to the community because of the help that I have received from others. I remember walking into that building and the first sensation that went through my mind was that everyone looked depressed. It was then that I saw this man sitting on a chair with his shirt tucked in his pants and his wavy grey hair brushed to one side. I sat down next to him and we started talking about his life, how he traveled around the world and lived for a few years in France. He was an interesting man, so I thought that I should come over more often to keep him company. The next week I arrived to the nursing home and when I approached him he tried to recognize me, but he didn't, it was then that I knew that he had Alzheimer's. It felt bad at first when he didn't remember me. I

[1] Pg 2, *2012 Alzheimer's Disease Facts and Figures,* Alzheimer's Association. March 2012. http://www.alz.org/documents_custom/2012_facts_figures_fact_sheet.pdf

[2] See Graph 1. in appendix.

thought he would be happy to see me again but after a while when you understand what the disease is you just hope to see a miracle so they can cure this devastating illness.

After a year, during my first year in *Bachillerato* students are required to write and carry out an investigation project about a theme of their choice. So I had to choose a subject that I could develop and expand, but I had no clue what to write about. I asked my dad for help and he told me that he was reading a book called *Inferno*, which is written my Dan Brown.[3] The book tells the story of Robert Langdon who is a professor in Harvard University and a crazy scientist. During the book he concludes that the overpopulation on Earth and the increase of population that will develop in the following years is such that it will wipe out the entire environment and all the natural resources in the planet. So, he creates a viral vector, which is genetically modified to sterilize humans, and aims it so it just affects 40% of the population. This viral vector will regulate the population level so humans can still live on Earth. After that, my dad told me "why don't you write about the use of viral vectors in some disease." The first thing that popped in my head was that old man in the nursing home, so that's what I choose.

This investigation project has as its starting point the assumption that viral vectors is going to be the definitive treatment of Alzheimer's disease. However, clinical trials data, scientific publications and opinions of scientists from recognized institutions will show us a different perspective. You will learn that viral vectors are only an instrument used in clinical trials on this disease and not a treatment in itself. Ultimately, I will dismantle my hypothesis and along the way I will point out what the scientific community is really doing in order to find "the cure" for Alzheimer's.

As you can see I've divided my Index in two parts. The first part is where I explain the disease and what it is about. The second part is where I explain what a viral vector is and its use in different treatments. During my project I

[3] *Inferno,* Dan Brown. Doubleday 2013.

talk about the interviews I've had and how they influence my conclusion and the progress of my investigation work. With the help of the appendix you will be able to compare the description with an image and look at the articles that I have used for the investigation.

# PART 1: Alzheimer's Disease

## Introduction:

The Alzheimer's disease is a neurodegenerative illness. It is a type of dementia[4], which causes memory lost, and destroys thinking skills. Alzheimer's is a progressive and irreversible disease, it worsens over time. This disease affects normally people that are 60 or older.[5]

In 1906 Dr. Alois Alzheimer[6] found that patients died after years of struggling with memory lost and disorientation. This fact made him perform a brain autopsy of these patients. He later identified brain cell abnormalities like patches (neurotic plaques) in the cerebal cortex and twisted bands of fiber (neurofibrillary tangles). This information is used today to diagnose Alzheimer's disease.

During the 1960's scientist related the cognitive problems with the number of plaques and tangles in the brain. From the 70's to the 90's the attention was focused on the understanding of the nerve cells in the brain of an Alzheimer's patient in order to find different drugs to treat the cognitive symptoms of the illness.

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[4] See glossary Nº1.

[5] *The Basics.* Alzheimer's Disease Education & Referral (ADEAR) Center. USA. June 2011. http://www.nia.nih.gov/sites/default/files/LaEnfermedaddeAlzheimer.pdf

[6] Alois Alzheimer, born in Markbreit, Bavaria on the 14 of June 1864 and died in Breslau, Silesia on the 19 of December 1915, was a German physician that published the first case of Alzheimer's. *Alois Alzheimer.* Wikipedia. June 2014. http://es.wikipedia.org/wiki/Alois_Alzheimer

However, in the last decades there has been a huge progress in the understanding of both genetic and environmental factors that lead to this disease. As well as the regions affect by this disease and the formation of patches and tangles in the brain. Not forgetting to mention the constant search of more effective drugs to kill the symptoms.[7]

## Symptoms:

Physiological changes start in the entorhinal cortex where the healthy neurons lose their efficiency and in the end they die. This process spreads towards the hippocampus, the region of the brain where we keep our memories. The increase of the dead neurons makes the regions affected shrink. In the final phase of the disease, the brain tissue suffers a significant shrunk.

At some point, the damage that is occurring in the brain has to reflect itself in the person. It's important to distinguish the different stages of the disease because in each stage the progress of the illness is different and the symptoms get worse.

Before the detection of the disease there are some early aspects that start to appear, such as difficulty for word finding and reasoning. The first stage of the illness is called the **mild stage**. In this stage memory loss worsens and the ill patient has a hard time with other cognitive abilities. For example, forgetting to pay the bills, getting lost, having some mood changes and repeating questions. These changes are evident and patients are often diagnosed during this stage.

After uncovering this disease, the next stage is called the **moderate stage** damage occurs in the region of the brain that controls the language, the reasoning and the conscious thought (temporal lobe). Memory loss grows worse and they

[7] *A History of Alzheimer's disease*, Clarksburg, United States of America. April 15 of 2014. http://www.brightfocus.org/alzheimers/about/understanding/history.html

will have problems recognizing people and difficulty with every day issues. They can start having hallucinations, paranoia and delusions.

By the **severe stage** of the illness, the brain has shrunk significantly and people with severe Alzheimer depend on a caretaker for help because they cannot communicate. At the end of this stage the ill patient might be in bed most of the time because the whole body starts to shut down.

Nonetheless, the important question here is what causes this devastating disease. Researchers don't absolutely know the explanation to the cause of Alzheimer's disease. Although they believe that there are some genetic, environmental and lifestyle factors that play a big role in this disease.

## Forms:

We could say that there are two different forms of this disease, the early-onset and late-onset Alzheimer. Neurologists agree that these two forms are essentially the same disease.

- **Early-onset** form is diagnosed before the age of 60. This form is really uncommon, with less then a 10% of all Alzheimer's cases[8]. Genetics play a big role in this form of Alzheimer, considering that early-onset Alzheimer is hereditary. The 23 human chromosome pairs contain all 30 thousand genes that code specific proteins. There are three rare genetic mutations that cause this form of Alzheimer's:

- Mutation on Chromosome 21, which contains the gene encoding for the production of the Amyloid Precursor Protein.

[8] *Early-onset Alzheimer's strikes families fast and ferociously,* Céleste Owen- Jones BBC. New York City, USA. 30 May 2012. http://www.bbc.com/news/magazine-18067401

- Mutation on Chromosome 1 & 14, which affect molecules called Presenilins related to the presence of Gamma Secretase.

- Mutation on Chromosome 19. People carrying this mutation has a certain variant of a gene, which controls the production of Apolipoprotein E (ApoE), meaning that they have a high risk of having Alzheimer's.

- **Late-onset** Alzheimer is the most common form of this illness and 90% of all Alzheimer's disease patients have this form. This type of Alzheimer is develops after the age of 60. "The causes of this form are not yet completely understood, but they likely include a combination of genetic, environmental, and lifestyle factors that influence a person's risk for developing the disease."[9]

## Diagnosis:

Scientist know that brain changes start 10 or 20 years before the person realizes that they have the disease and if it's detected early some treatments for Alzheimer's are likely to be more effective. But how do we know if we already have the disease or in the future we have a high probability of having it?

The only way doctors know certainly that the person has Alzheimer's is by examining the brain tissues during the autopsy and looking at their clinical course. However, memory loss is one of the first symptoms of Alzheimer's, it's also a common symptom for other cognitive diseases. Doctors have several methods to determine if Alzheimer's causes these symptoms:

[9] *Late-Onset Alzheimer's Disease.* Alzheimer's Disease Education & Referral (ADEAR) Center. USA. June 2011

- They will ask them questions about past medical problems, difficulties executing everyday activities and mood changes.
- They will perform memory, language and counting test.
- They will carry out blood and urine test.
- They will take brain scans to determine if Alzheimer's is the cause or if there is something else that causes these symptoms, such as tumors, strokes or Parkinson's disease[10].

These tests will be performed and observed throughout the clinical course of the patient.

Recently, there is an investigation being done to find **Biomarkers**[11] that will allow us to diagnose before hand if a person will have the disease and improve and broaden their knowledge of the causes and changes in the disease. This could help develop new treatments and drugs that will mitigate and cure this illness. I got the chance to tour around *La Fundación Pasqual Maragall* and interview Mr.Juan Domingo Gispert López, which works in this foundation. During the interview he tells me that the foundation is working and investigating on Biomarkers.[12]

## Cause:

The origin or the cause that produces this disease is unknown, but scientists know for sure that aging is a big factor that contributes in having this disease. There are several theories that try to explain what is the cause of neurodegeneration.

[10] See glossary Nº 2.

[11] See glossary Nº 3.

[12] See interview in appendix.

- **The Cholinergic theory:** This theory explains that the root of this disease comes from a deficiency in the synthesis of one of the neurotransmitter[13] in the cholinergic system called acetylcholine. The acetylcholine (ACh) is synthesized in the interior of the presynaptic neuron with the help of an enzyme called Choline acetyltransferase (ChAT) and kept in vesicles inside the neuron. When there is a nerve impulse, acetylcholine is resealed into the synapse and interacts with the cholinergic receptors located in the postsynaptic neuron membrane. Acetylcholine acts on two different types of receptors: nicotinic receptors and muscarinic receptors and thanks to this interaction the nerve impulse is transmitted.[14] If there is a reduction in the synthesis of this neurotransmitter, nerve impulses will not be transmitted and the neurons will eventually die.

- **The Amyloid theory:** This theory says that the accumulations and deposits of a protein called beta-amyloid[15] (Alphabeta) is the main cause of this disease. The amyloid precursor[16] protein is a protein that is processed by two enzymes called Alpha Secretase and Gamma Secretase in physiologic conditions[17]. That cleavage process produces the non-pathologic P3 peptide.

  However, in ill conditions the amyloid precursor protein is processed by two different enzymes: Beta-secretase and Gamma secretase. The combined action of those secreatases develops a protein called **Beta-amyloid**.[18] Beta-amyloid in normal conditions isn't harmful but when the

[13] See glossary Nº 4.

[14] See Fig. 1 in appendix.

[15] See glossary Nº 5.

[16] See glossary Nº 6.

[17] See glossary Nº 7.

[18] See Fig. 2 in appendix.

pH[19] changes, oxidative stress increases or it starts interacting with other proteins it suffers a change in its structure and aggregates.[20] The beta-amyloid that is aggregated accumulates on the exterior part of the neuron and forms the senile plaques[21].

- **The Tau theory:** This theory states that the accumulation of the tau protein forms what is called neurofibrillary tangles and are known to be one of the primary causes of Alzheimer's disease.

  The tau protein is a protein that stabilizes microtubules in the neuron and in physiologic conditions binds with a tubulin[22]. This fusion is controlled by two enzymes called protein kinase and phosphatase. The first enzyme adds phosphate groups to the tau protein while the other one removes phosphate groups.

  Nevertheless, in pathological conditions there is a hyper phosphorylation of the tau protein causing it to not bind with the tubulin. After that the tau protein will start binding with other tau proteins causing the formation of neurofibrillary tangles that disintegrate the microtubules in the neuron. When this happens the neurons are not capable of communicating and later the may result in the death of these cells. [23]

- **The Inflammatory hypothesis:** This theory, in the passed years, has achieved some eminence in the studies of Alzheimer's because we know that inflammatory molecules are often entailed with senile plaques. Nevertheless, this theory states that this disease appears as a

[19] See glossary Nº 8.

[20] See Fig. 3 in appendix.

[21] See Fig. 4 in appendix.

[22] See glossary Nº 9.

[23] See Fig. 5 in appendix.

consequence of brain inflammation, which forms abnormal metabolites[24] from normal brain molecules. This inflammation process, that creates metabolites, is triggered by various stimuli such as, infections or traumatic head injuries.

However, the problem starts when these abnormal metabolites along with cytokines[25] course through the brain modifying beta-amyloid proteins and causing them to form into insoluble beta-amyloid plaques.[26]

- **The Oxidant hypothesis:** This theory says that free radicals[27] accumulate in the brain and affects the nerve cells by altering the protein structure and DNA that will result in the lost of the cells functions, which may contribute in Alzheimer's disease.

  When molecules split as result of the bodies metabolism free radicals are produced, these molecules react with other compounds to gain stability. But what they actually do is somehow steal electrons from this other compound, setting off a cascade of disruptions that will affect living cells as well as neurons. In normal situations the body regulates free radicals on its own but if there is a lack of antioxidants or they're just unavailable in one's diet and if there is an excessive production of free radicals damage will occur.

  External agents (infections, herbicides, radiation, etc.) can accelerate the production of free radicals.

[24] See glossary Nº 10.

[25] See glossary Nº 11.

[26] *Taming the scientific story of AD.* The Myth Of Alzheimer's. Peter J. Whitehouse. St.Martin's. USA. 2008

[27] See glossary Nº 12.

- **Other hypothesis:**

    i. ECD (Excitatory Cell Death) theory: Neurons die because of the excess stimulation conducted by glutamate or other neurotransmitter.

    ii. Infectious Disease theory: It has been claimed that there is evidences of viruses (prions) and other infections in the brains of patients with AD.

    iii. Diabetes theory: Disturbances in glucose metabolism produces vascular damage that affects low flow to neurons

## Therapeutic Routes:

We are investing our resources, our time, and our hope in the pursuit of a cure that may never arrive for a disease that we cannot diagnose or effectively treat. During my investigation I have read and spoken to investigators that work in this area of this disease. While researching I saw that many treatments had different therapeutic routes, so I have divided them depending on the therapeutic route that they are based on.

- **Genetics and Molecular Medicine:** The new century brought a new hope for future tests and AD therapies based on molecular genetics and biology thanks to the hard work of scientists that in April of 2003 published the sequencing of the human genome.[28] In this group of

[28] *Human Genome Project.* Wikipedia. 22 of November 2014. http://en.wikipedia.org/wiki/Human_Genome_Project#Findings

treatments there are different drugs and therapeutic routes that are related to genetics and molecular medicine.

I. Inhibitors: Some treatments act as inhibitors meaning that they decrease the rate or they stop completely a reaction:

i. Cholinesterase: Cholinesterase is an enzyme found primarily at nerve endings that catalyzes the hydrolysis, or decomposition, of acetylcholine. Acetylcholine is a neurotransmitter essential for learning and memory. Researches revealed, the loss of cholinergic nerve cells in a part of the brain called the cholinergic basal forebrain[29] is associated with de symptoms of dementia.

We don't know exactly why these cells in the basal forebrain die, or how to prevent their death. So current treatments focus on inhibiting cholinesterase, the enzyme that breaks down acetylcholine in the brain. The family of FDA[30] approved cholinesterase inhibitors are tacrine, donepezil, rivastigmine, and galantamine. They were created as a result of this research, which came to be known as "the cholinergic hypothesis" which I have stated above in this investigation project.

Although these inhibitors demonstrate tiny improvements on cognitive and global function in some patients with mild to moderate Alzheimer's, and may work in some severe and serious cases as well, they do not work for others. They may have some side effects[31] that include are

[29] See Fig. 6 in appendix.

[30] See in glossary Nº 13.

[31] See in glossary Nº 14.

gastrointestinal upset, nausea, vomiting, diarrhea, and muscle cramps as well as sleep disturbances, insomnia, and nightmares.

ii. Memantine: A recent addition to the list of FDA approved drugs is Mematine. It acts in a different way and through a different mechanism than cholinesterase inhibitors. Memantine's action involves the inhibition of glutamate. Glutamate is an omnipresent present neurotransmitter in the cerebral cortex. Experimental research in animals has shown that if glutamate receptors are over stimulated by the abundance of glutamate, neurons may die. Memantine blocks the effects of glutamate. However, there is no clinical evidence to demonstrate this effect. There are some side effects such as headache, constipation, dizziness, and agitation.

II. Gene Therapy: this treatment involves changing the genes from your body's cells to cure an illness:

i. Stem Cells: Stem cells are undifferentiated cells that have the potential to act as a repair system. They can be used to build specific tissues or organs. They are taken from the blood of the umbilical cord and through manipulation they may be potentially be grown into neurons. These stem cells are implanted to recover function of memory, they would have to form complicated connections with neurons and this seems like it is not 100 % feasible.

ii. Nerve Growth Factor Fibroblasts: This possible treatment says that implanting genetically modified cells in the brain that are capable of producing nerve growth factor (NGF),

a protein that prevents neuronal death and stimulates cell function, can cure Alzheimer's.

Skin cells called fibroblasts are taken from patients. These cells are infected with a virus that has a copy of the nerve growth factor gene inserted into its genetic material. The fibroblast serves as a pump producing NGF. The risk of this treatment is uncontrolled growth of brain cells as occurs in brain cancer.

III. Drugs: Pharmaceutical drugs can also help prevent the death of neurons:

i. Cholinergic drugs: This drug stimulates the neuronal synapsis and contains compounds as nicotine and acetylcholine. This means that is produces the same effect as acetylcholine.

ii. Neurochem: This drug has as primary target the amyloid protein, preventing it from forming into beta-amyloid plaques (BAP) deposits and filtering it out of the body. This seems like it can have some success on mild AD patients, but not with people with moderate disease.

iii. Flurizan: This drug also has as primary target the amyloid protein. It is a compound that binds to the gamma secretase and causes it to produce shorter forms of beta-amyloid.

IV. Plasmapheresis: I will go in depth in this treatment because it is a treatment done here in Barcelona and I got the chance to speak to a professional about it. Plasmapheresis is a Greek word, *plasma* meaning something formed and *aphaeresis* meaning

taking away.[32] Therefore, this is an extracorporeal[33] procedure where blood is removed from the patient's body and then the blood cells are separated from the plasma. After that, the blood cells are returned to the body with new and fresh plasma or albumin.

You might ask yourself how do doctors separated every blood cell from the plasma. There are two ways to accomplish this: centrifugation or semipermeable membranes.[34] Centrifugation is a process that takes into account the different specific gravities of each blood cell like red cells, white cells, platelets and plasma. [35] The use of semipermeable membranes to separate all the blood cells from the plasma is quite interesting. A semipermeable membrane as the word says, it's a membrane that allows some molecules to pass through but not others, so this process takes advantage of the different particle sizes of the blood cells. We can say that it is like a filtration of the plasma to differentiate the cellular components of the blood.[36]

This procedure is used as a therapy of autoimmune disorders. These diseases are caused by an abnormal immune response of the body against substances that are normally present in the body.[37] They remove any harmful autoantibodies from the blood,

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[32] Plasma. (n.d.). Dictionary.com Unabridged. Retrieved November 03, 2014, from Dictionary.com : http://dictionary.reference.com/browse/plasma

[33] See glossary Nº 15.

[34] *Plasmapheresis Periprocedual Care.* Elliot Stieglitz. Medscape. 13 of December 2013: http://emedicine.medscape.com/article/1895577-periprocedure

[35] See Fig. 7 in appendix.

[36] See Fig. 8 in appendix.

[37] *Autoimmune Disease*. Wikipedia. November 2, 2014: http://en.wikipedia.org/wiki/Autoimmune_disease

but the production of those antibodies will have to be stopped by medication.

Like every other medical therapy, there are lots of risks and complications that can happen during the procedure and that must be taken into account before and during the process of this therapy:

a) The first one that doctors can come up to is *anaphylaxis,* during the reinfusion of patient with the fresh plasma. Anaphylaxis is a rapidly progressing, life-threatening allergic reaction that can proceed to a complete airway obstruction, shock or death. The treatment involves injecting adrenaline, which compresses blood vessels and acts against the effects of histamine.[38]

b) Another risk that can come up is a bacterial infection or blood clots when a central venous catheter[39] [40] is used.

c) Cramps and numbness may be caused by a reaction to the citrate anticoagulant used during plasmapheresis. Some patients with a disabled kidney function may require some drugs for the effects of the citrate anticoagulant.[41]

d) Patients can experience symptoms of hypocalcaemia, presence of low serum calcium levels in the blood or hypomagnesaemia, low blood magnesium levels, during and

[38] See glossary Nº16.

[39] See glossary Nº 17.

[40] See Fig. 9 and 10 in appendix.

[41] See glossary Nº 18.

after the treatment and can be treated by oral replacements of calcium and magnesium.

e) Patients can become hypothermic[42] during the therapy and they should be warmed up appropriately.

f) Patients can experience hypotension[43] as a result of the transfusion and should be monitored at all times to minimize unexpected falls.

g) Patients can become thrombocytopenic, meaning they have low platelet levels and hypofibrinogenemic, meaning a severe hemorrhagic state due to an inability of the blood to clot.

h) Like all procedures it may cause a mild allergic reaction, leading to fever, chills and rashes.[44]

During my investigation I got in contact with *Barcelona Alzheimer Treatment and Research Center*, which is a nonprofit organization that researches, does counseling, write documentation and aims to inform the families and the patients that are suffering from this type of dementia and of course the welfare of the people. I got to talk to Mrs. Isadora Jimenez the communication manager from the foundation. She told me that the clinical trials unit was conducting several studies with monoclonal antibodies and a trail about

[42] See glossary Nº 19.

[43] See glossary Nº 17.

[44] *Plasmapheresis*. The Free Dictionary. 2008: http://medical-dictionary.thefreedictionary.com/plasmapheresis

plasmapheresis. I decided to find out more about the second therapy.

In this therapeutic plasmapheresis, a volume of plasma is extracted from the patient and substituted by a 5% albumin solution called *Human Albumin Grifols 5%*. As we know beta-amyloid peptide accumulates on the exterior part of the neuron and forms the senile plaques involved in memory function. Considering that 90% of beta-amyloid circulates in the plasma and it is linked to albumin, the extraction of the beta-amyloid with plasmapheresis could encourage a mobilization of the beta-amyloid in the brain and consequently lead to an improvement of all the cognitive functions of the patient.

This pilot study was performed on patients with mild Alzheimer's disease and it revealed that there was clinical stabilization after the plasmapheresis was performed and that plasma levels of $A\beta_{40}$ and $A\beta_{42}$ presented a saw-tooth pattern associated with the plasma exchanges.

However, as Mr. Juan Domingo Gispert from the Pasqual Maragall Foundation said in my interview, this treatment is unethical because people with this illness is desperate for a cure, and 50% of the patients treated with this therapy has infections. He does not recommend this therapy at all.

- **<u>Alternative Treatments</u>:**

I. <u>CAM's:</u> For a long time, many people around the world have relied on so-called complementary and alternative medicines for their basic health care. A wide range of different treatments, including; diet, nutritional products, herbal supplements, and other mediations, are providing therapeutic options. Integrative

medicines tend to focus on prevention and maintenance of health rather than the exclusive treatment of disease. Some of these alternative treatments are also used for Alzheimer's; chiropractic, yoga and meditation, acupuncture and the use of herbs.

## PART 2: Viral Vectors

First of all, before explaining what a viral vector is, we will have to know what a virus is. A virus is an infective agent that is only capable of replicating itself inside living cells. They use the cell machinery to reproduce more viruses. Virions, the infectious virus particles, consist of three parts. The first one is the nucleic acid, they may be RNA or DNA and single or double-stranded. They must have a protein coat, known as the capsid, that surrounds the genetic material (RNA or DNA) for protection. Some of them will also have a lipid membrane that surrounds the protein coat.

### Structure of a Virus:

Viruses can have different shapes too. Some of them can have a simple helical or icosahedral shape, and others can have a complex structure.

- Viruses with a helical structure, the capsomers are stacked forming a hollow tube and protecting the genetic material that is coiled in it.[45] An example of a helical virus is the tobacco mosaic.[46]

[45] *El Virus*, M.Costa, D. Bueno, M. Formiga, R. Grau, M. Llobera, J.M. Llort, J. De Manuel, J. Molina, P.Monserrat, T. Padrosa. Editorial Teide. Barcelona.

[46] See Fig. 11 in appendix.

- Viruses with an icosahedral shape, the capsomers are placed as an icosahedron[47]. An adenovirus is an example of an icosahedral virus.[48]

- Viruses with a complex structure are formed by two parts. The first part is the head, where you can find the nucleic acid. The second part is the tail, which is composed by a helical structure and fibers that look like legs and they act as a "molecular syringe" to inject the genetic material of the virus.[49]

## Life Cycle of a Virus:

As I said before, some scientists agree that viruses are not considered a living organism because they rely on a host cell to reproduce. The replication cycle of a virus has seven stages; attachment (also called adsorption), penetration, uncoating, genome replication, transcription and translation, budding and lysis.

1. In the first stage, the virus attaches to the cell that it is going to infect. Viruses have specific proteins on the capsid that bind with the receptors of the host cell.[50] The specificity of the receptors determines the range of cells that can act as a host to a virus. Meaning that one virus cannot infect all the cells in a body, only the ones that bind with the viral attachment molecule.

2. Penetration is the second stage of the replication cycle. In this stage the virus or the nucleic acid of virus will enter the host cell. However, it depends if it's an enveloped virus or a nonenveloped virus.

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[47] See glossary Nº 21.

[48] See Fig. 12 in appendix.

[49] See Fig. 13 in appendix.

[50] See Fig. 14 in appendix.

i. If its and enveloped virus, it will enter the host cell by receptor-mediated endocytosis where the virus is “ingested” by the cell.

ii. If it’s a noneveloped virus, the lipid bilayer of the virus fuses with the lipid bilayer of the cell membrane. This is called membrane fusion.[51]

3. Uncoating is the third stage and it is where the capsid is detached and the viral genome is released. The viral or host enzymes break up the capsid.

4. Replication is the most important part of all. This stage consists of the multiplication of the viral genome. The method varies depending on the virus.

    i. If it’s a DNA virus the replication takes place in the host cells nucleus. The viral DNA is inserted into the host cell genome and then uses the host cell machinery to replicate.

    ii. If it’s a RNA virus replication will take place in the cytoplasm.

    iii. If it’s a Reverse Transcribing Virus replication takes place first in the cytoplasm and then in the nucleus. The virus inserts the strands of RNA with three types of enzymes that will be used for the replication of the viral genome. The first step is converting viral RNA to DNA with the help of a reverse transcriptase[52]. After that integrase[53] grabs the viral DNA and takes it to the house cell nucleus where it inserts the viral DNA into the host cell genome.

[51] See Fig. 15 in appendix.

[52] See glossary Nº 22.

[53] See glossary Nº23.

5. The fifth stage is transcription and translation of the host cell genome. In this stage the proteins are formed and are used to build and create the structural parts of a virus, like the capsid.
6. Budding is the sixth stage of the life cycle of a virus. In this step the parts of the virus created earlier are acquired.

7. Lysis is the last stage. This is where viruses escape from the host cell by rupturing the cell.

## Types:

There are five kinds of viral vectors, but I will discus three of them because these three are normally used in medical treatments.[54]

- *Retrovirus* is a type of virus in the *Retroviridae* family. It duplicates in a host cell but the difference between this type of virus and the others is that retroviruses replicate through a process called reverse transcription and they only infect dividing cells. Retroviruses have an easy structure; they are a single-stranded RNA virus and the envelope[55] is made up of lipids and glycoproteins encoded by the env gene.

- *Lentivirus* is a subclass of the *Retroviridae* family. However, the difference between a Lentivirus and a Retrovirus is that a Lentivirus only infects non-dividing cells and they are capable of delivering a high amount of viral RNA in to the host cell. This is why scientists use Lentiviruses in gene delivery.

- *Adenovirus* is a member of the *Adenoviridae* family. Adenoviruses have no outer lipid bilayer; this means they have no envelope. They have an

[54] See Graph. 2 in appendix.

[55] See glossary Nº24. See Fig. 16 in appendix.

icosahedral capsid and inside it you can find the double stranded DNA genome. [56]

## Application:

### Gene Therapy

Gene therapy is a technique that uses genes to treat or prevent a disease.[57] Scientists are testing three techniques that might allow doctors in the future treat or even prevent a disease without surgery or drugs. The first form is by replacing a mutated gene with a healthy copy of the gene. The second form is inactivating a mutated gene that is not working properly and the third form is introducing a new gene in to the patient's body so it helps treat the disease.

Nevertheless, viruses are used in gene therapy as vehicles to carry the healthy genes to the human cells. Because viruses introduce their own genes into our cells and reprogramming them to duplicate, researchers think that if we modify the viruses' genetic information with the new genetic information that will treat a disease, we will be able to cure the patient.

Both adenoviruses and retroviruses[58] are often used in gene therapy but they both have a different way of integrating their genetic material to a cell. On one hand, retroviruses integrate their genome, with the new healthy gene, into a chromosome in a human cell.[59] On the other hand adenoviruses introduce their

[56] See Fig. 12 in appendix

[57] *What is gene therapy?*, May 26, 2014: http://ghr.nlm.nih.gov/handbook/therapy/genetherapy

[58] See Graph. 3 in appendix.

[59] See Fig. 17 in appendix.

DNA into the nucleus of the cell but the DNA does not integrate into a chromosome.[60]

There are numerous problems and risks related to this application of viral vectors in gene therapy. First of, we know that viruses can infect more then one type of cell, so when we use viral vectors to carry genes into the body, they can infect healthy cells and reproductive cells. If they deliver the gene to a reproductive cell it could produce changes that will be passed on and inherited by the patients children. Another risk is that the new gene could be inserted in the wrong location in the DNA and this could possibly cause a harmful mutation to the DNA or it can even cause cancer. However, researchers and scientist are fixing these potential problems.[61]

## Vaccines

The beta-amyloid hypothesis has led to most companies that study Alzheimer's to create methods that prevent the formation, that dissolve and that improve the clearance of beta-amyloid in patients. A rather surprising approach has concentrated on the production of amyloid antibodies to promote the removal of beta-amyloid in the brain. It was theorized that a vaccine could increase the body's production of amyloid antibodies, which would help prevent the widespread buildup of plaques.

First of, vaccination consists of "stimulating the immune system with an infectious agent, or components of an infectious agent, modified in such a manner that no harm or disease is caused, but ensuring that when the host is confronted with that infectious agent, the immune system can adequately neutralize it before it causes any ill effect."[62] Vaccination has been approached by two ways. The first

[60] See Fig. 18 in appendix.

[61] *Viral Vectors*: http://www.genetherapynet.com/viral-vectors.html

[62] *DNA Vaccines*. World Health Organization: http://www.who.int/biologicals/areas/vaccines/dna/en/

one is introducing a specific antigen that later the immune system will react directly to it. The second way is by introducing an agent, which is reduced infectively, that will replicate in a host cell and will synthesize the antigens that will stimulate the immune system.

In 2002, a group of Japanese investigators led by Takeshi Tabira and Hideo Hara[63] [64]tested a new vaccine to fight against Alzheimer's. They applied it on transgenic mice, which were modified to have the amyloid precursor protein, and the vaccine was administered orally. They used an adeno-associated virus as a vector to introduce a beta-amyloid complementary DNA[65]. This induced the expression and secretion of beta-amyloid in the Intestine in the transgenic mice.

The expectation of this investigation was to promote the production of antibodies in response of this damage. The results were at first prominent as the serum antibodies levels increased as months went by, there was a significant reduction of amyloid deposits and the mice showed an improvement in memory lost. So after the great results they started a clinical trial with humans and in March of that same year they had to abandon the trial because of complications. 18 patients out of 298 appeared to have meningoencephalitis[66] caused by autoimmune T cells that reacted to beta-amyloid. After one of the patients deaths, the investigators did an autopsy and determined that that there was an effective clearance of beta-amyloid and also a production of antibodies against senile plaques.

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[63] Pg. 265, Chapter 26. *Advances in Alzheimer's and Parkinson's Disease.* Abraham Fisher. Maurizio Memo. Fabrizio Stocchi. Israel Hanin. Springer. 2008.

[64] See Article. 1 in appendix.

[65] See in glossary Nº 25.

[66] See in glossary Nº 26.

The conclusion for the investigators was: " Beta-amyloid vaccination seems to be a promising way to prevent the onset and progression of Alzheimer's, if the T cell-mediated side effect is minimized."

This takes us to another investigation done by a biotech firm called Elan. This firm developed a trail with passive immunization. This therapy involves giving premade antibodies to individuals rather than having the individual's body produce them as occurs with active immunization. The positive part of this new therapy is that it decreases the risk of meningoencephalitis.

## Results

As you can see in Graph. 4 in the appendix, in the past five years there has only been 5 clinical trials done with viral vectors. While with beta-amyloid 64, protein TAU 29, vaccines 51, biomarkers 152, etc. You can see clearly that there is a big difference and this may mean that the use of viral vectors in the treatment of Alzheimer's may not be the right therapeutic route to cure it. On Graph. 5 in the appendix you can see what type of clinical trials have been done or are in progress in Spain. As you can see viral vectors are not in one of those subdivisions.

We can say that the majority of scientists are not focusing on the use of viral vectors for a treatment of Alzheimer's because of the collateral affects and the high risk for patients to develop infectious diseases or mutations in their chromosomes.

## Conclusion:

This research project has been aimed to demonstrate that the use of viral vectors is the future and most successful treatment for the cure of Alzheimer's. To demonstrate this I had to investigate the causes of this disease, the clinical trials

and the current treatments that are performed today. From all the possible causes of this illness, the one that stands out and is the most common in different clinical trials is the investigation of the aggregation of beta-amyloid in the synaptic cleft, which causes the death of the neurons.

During my interviews with the *Pasqual Maragall Foundation* and the *Barcelona Alzheimer Treatment and Research Center* (ACE) I have confronted my hypothesis and in both cases they have rejected the use of viral vectors compared to other treatments. ACE did not show great interest in developing the reasons. I do not know if it is because it's new line of research or as Mr. Juan Domingo Gispert briefly noted in the interview, you can't control the direct effects of the cancellation of the production of this protein, because it also has a beneficial physiological function, or the high risk of undesirable side effects. I am convinced that generating antibodies from other parts of the body could control this last effect, like some researchers at the *National Institute of Biomedical Innovation of Japan* have been getting from the intestine, and have even patented them.[67] But, I understand that the fact that you cannot "partially" vaccinate someone is a roadblock for this research line.

In the course of this project, I have been able to find out that there are different theories and that each one does not exclude the others. I found out that there is not one cause that triggers this disease, but more than one.

This investigation project has helped in a lot of ways. First off, I have learned many things about this disease that I did not know before. Because it's a really complex illness it was hard to understand the different theories and the treatments but in the end I think I did a good job. Also, I think that this project will help in the future. Meaning that in the universities they will ask you to do these project all the time, so I will have an advantage over my colleagues because I will know how to present and write one up. To finish up I think that the only problems that I had was that there was not a lot of information about clinical trial using viral

[67] Pg. 7. *Recombinant Adeno-Associated Virus Vector For Treatment of Alzheimer Disease.* Takeshi Tabira. Hideo Hara. January 2009.

vectors and the three interviews that I did, Mrs. Isadora Jimenez from the ACE and professor Francisco Muñoz form the Pompeu Fabra University did not give the reason why viral vectors could not be a possible treatment.

Recently, I attended an Open Day at the College of Amsterdam where I had the opportunity to hear a lecture entitled: “The Human body expires after 40 years” by the professor Dr. Jan Hindrik Ravesloot, where the conclusion was that Human beings are worn out after 40 years on planet earth. The fact that our species longevity increases over the years entails a natural process of aging of our cells, including neurons. The neurons functions or roles are affected by different “aggressions” along our life and I wonder if the aging of the brain can be considered a disease.

I read some reflections by Dr. Peter Whitehouse founder of the University Memory and Aging Center of Cleveland (Ohio) where he says that Alzheimer’s represents our culture attempt to make sense of a natural process: brain aging, which we cannot control.

In my personal opinion we are far away from understanding this disease and I think it will take us a while to discover the ultimate cause of this illness. So I would agree with some investigators that say It looks like Alzheimer’s cannot be differentiated from normal aging, and that we shouldn’t look for the “Fountain of youth”, in the meantime we should invest our money more wisely by putting them toward prevention and care rather than predominantly in cure. We must integrate more therapies that engage patients with other human beings and with nature. Human connections have an enormous potential.

## Glossary

1. **Dementia:** An overall term that describes a group of symptoms that are linked to the deterioration of mental abilities and that affects a daily life of a person. The American Heritage® Stedman's Medical Dictionary.

Houghton Mifflin Company. 08 Sep. 2014. <Dictionary.com http://dictionary.reference.com/browse/dementia>.

2. **Parkinson**: Disorder of the central nervous system. Trembling, trouble walking and maintaining balance are some evident symptoms of this disease. National Institute of Neurological Disorders and Stroke. http://www.ninds.nih.gov/disorders/parkinsons_disease/parkinsons_disease.htm. Accessed Aug. 8, 2013.

3. **Biomarkers:** a distinct biochemical, genetic, or molecular characteristic or substance that is an indicator of a particular biological condition or process. (n.d.). Dictionary.com Unabridged. Retrieved November 25, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/biomarkers

4. **Neurotransmitter**: brain chemicals that communicate information throughout our brain and body. *What are Neurotransmitters?.* Neurogistics. https://www.neurogistics.com/TheScience/WhatareNeurotransmi09CE.asp

5. **Beta-amyloid**: Protein made out of 40 or 42 amino acids. *Beta Amyloid.* Wikipedia. November 2014. http://en.wikipedia.org/wiki/Beta_amyloid

6. **Precursor**: In biochemistry, the term "precursor" is used more specifically to refer to a chemical compound preceding another in a metabolic pathway. *Precursor (chemistry).* Wikipedia. March 2014: http://en.wikipedia.org/wiki/Precursor_(chemistry)

7. **Physiologic conditions**: Something that is normal, that is due neither to anything pathologic nor significant in terms of causing illness. *Definition of physiologic.* Medicine Dictionary. March 2012: http://www.medterms.com/script/main/art.asp?articlekey=7911

8. **pH**: is a measure of the acidity or basicity of an aqueous solution. Solutions with a pH less than 7 are said to be acidic and solutions with a pH greater than 7 are basic or alkaline. *pH.* Wikipedia. August 2014: http://en.wikipedia.org/wiki/PH

9. **Tubulin**: A globular protein that is the structural constituent of microtubules. tubulin. (n.d.) *The American Heritage® Medical Dictionary*. (2007). Retrieved September 28 2014 from http://medical-dictionary.thefreedictionary.com/tubulin

10. **Metabolites:** A product of metabolic action. (n.d.). Dictionary.com Unabridged. Retrieved November 24, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/metabolites

11. **Cytokines:** Any of several regulatory proteins that are released by cells of the immune system and act as intercellular mediators in the generation of an immune response. (n.d.). The American Heritage® Science Dictionary. Retrieved November 24, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/cytokines

12. **Free Radical:** An atom or group of atoms containing at least one unpaired electron and existing for a brief period of time before reacting to produce a stable molecule. free radicals. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved November 24, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/free radicals

13. **FDA:** abbreviation that stand for *Food and Drug Administration*: a federal agency responsible for monitoring trading and safety standards in the food and drug industries. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved November 25, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/fda

14. **Side Effects:** any effect of a drug, chemical, or other medicine that is in addition to its intended effect, especially an effect that is harmful or unpleasant. (n.d.). Dictionary.com Unabridged. Retrieved November 25, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/side effects

15. **Extracorporeal:** outside the body. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved November 03, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/extracorporeal

16. **Histamine:** an amine formed from histidine and released by the body tissues in allergic reactions, causing irritation. It also stimulates gastric secretions, dilates blood vessels, and contracts smooth muscle. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved November 03, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/histamine

17. **Central Venous Catheter:** also called a central line, is a long thin flexible tube used to give medecines, fluids or blood products over a long period of time. *Central Venous Catheters- Topic Overview.* Web Med. December 14, 2012: http://www.webmd.com/pain-management/tc/central-venous-catheters-topic-overview

18. **Anticoagulant**: substance that delays or prevents the clotting of blood. (n.d.). The American Heritage® Stedman's Medical Dictionary. Retrieved November 03, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/anticoagulant

19. **Hypothermic:** an abnormallly low body temperature. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved November 03, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/hypothermic

20. **Hypotension:** decreased or lowered blood pressure. (n.d.). Dictionary.com Unabridged. Retrieved November 03, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/hypotension

21. **Icosahedron**: An icosahedron is a polyhedron with twenty triangular faces. icosahedron. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved September 08, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/icosahedron

22. **Reverse transcriptase**: It's an enzyme used to generate DNA from RNA and is needed for the replication of retroviruses. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved September 16, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/reverse transcriptase

23. **Integrase**: It's an enzyme produced by a virus that integrates the viral genetic material into the DNA of the infected cell. *Integrase*. Wikipedia. July 2014. http://en.wikipedia.org/wiki/Integrase

24. **Envelope**: An envelope is a membrane made out of lipids and glycoproteins that protect the capsid. envelope. (n.d.). The American Heritage® Stedman's Medical Dictionary. Retrieved December 07, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/envelope

25. **Complementary DNA:** Single-stranded DNA synthesized in the laboratory using messenger RNA as a template and the enzyme reverse transcriptase. Complementary DNA is used for many purposes such as mapping chromosomes, creating clones, and sequencing genes. (n.d.). The American Heritage® Science Dictionary. Retrieved November 23, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/complementary dna

26. **Meningoencephalitis:** Inflammation of the brain and its membranes. Also called *cerebromeningitis* **,** *encephalomeningitis* **.** (n.d.). The American Heritage® Stedman's Medical Dictionary. Retrieved November 23, 2014, from Dictionary.com website: http://dictionary.reference.com/browse/meningoencephalitis

## Bibliography


- Pg. 2, *2012 Alzheimer's Disease Facts and Figures,* Alzheimer's Association. March 2012. http://www.alz.org/documents_custom/2012_facts_figures_fact_sheet.pdf

- *Inferno,* Dan Brown. Doubleday 2013.

- *The Basics.* Alzheimer's Disease Education & Referral (ADEAR) Center. USA. June 2011. http://www.nia.nih.gov/sites/default/files/LaEnfermedaddeAlzheimer.pdf

- *Alois Alzheimer.* Wikipedia. June 2014. http://es.wikipedia.org/wiki/Alois_Alzheimer

- *A History of Alzheimer's disease*, Clarksburg, United States of America. April 15 of 2014. http://www.brightfocus.org/alzheimers/about/understanding/history.html

- *Early-onset Alzheimer's strikes families fast and ferociously,* Céleste Owen- Jones BBC. New York City, USA. 30 May 2012. http://www.bbc.com/news/magazine-18067401

- *Late-Onset Alzheimer's Disease.* Alzheimer's Disease Education & Referral (ADEAR) Center. USA. June 2011

- *Taming the scientific story of AD.* The Myth Of Alzheimer's. Peter J. Whitehouse. St.Martin's. USA. 2008

- *Human Genome Project.* Wikipedia. 22 of November 2014. http://en.wikipedia.org/wiki/Human_Genome_Project#Findings

- Plasma. (n.d.). Dictionary.com Unabridged. Retrieved November 03, 2014, from Dictionary.com : http://dictionary.reference.com/browse/plasma

- *Plasmapheresis Periprocedual Care.* Elliot Stieglitz. Medscape. 13 of December 2013: http://emedicine.medscape.com/article/1895577-periprocedure

- *Autoimmune Disease*. Wikipedia. November 2, 2014: http://en.wikipedia.org/wiki/Autoimmune_disease

- *Plasmapheresis*. The Free Dictionary. 2008: http://medical-dictionary.thefreedictionary.com/plasmapheresis

- *El Virus*, M.Costa, D. Bueno, M. Formiga, R. Grau, M. Llobera, J.M. Llort, J. De Manuel, J. Molina, P.Monserrat, T. Padrosa. Editorial Teide. Barcelona.

- *What is gene therapy?*, May 26, 2014: *http://ghr.nlm.nih.gov/handbook/therapy/genetherapy*

- *Viral Vectors*: http://www.genetherapynet.com/viral-vectors.html

- *DNA Vaccines*. World Health Organization: http://www.who.int/biologicals/areas/vaccines/dna/en/

- Pg. 265, Chapter 26. *Advances in Alzheimer's and Parkinson's Disease.* Abraham Fisher. Maurizio Memo. Fabrizio Stocchi. Israel Hanin. Springer. 2008.

- Pg. 7. *Recombinant Adeno-Associated Virus Vector For Treatment of Alzheimer Disease.* Takeshi Tabira. Hideo Hara. January 2009.

- *Genetics in Alzheimer's.* HBO Documentary Films, the National Institute on Aging at the National Institutes of Health in association with the Alzheimer's Association. http://www.alz.org/research/video/video_pages/genetics_in_alz.html

- *The Search for Alzheimer's Causes and Risk Factors.* Alzheimer's Association National Office. Chicago. 2014. http://www.alz.org/research/science/alzheimers_disease_causes.asp

- *Alzheimer's Disease Education and Referral Center.* National Institute on Aging. 2012. http://www.nia.nih.gov/alzheimers/publication/2011-2012-alzheimers-disease-progress-report/

- *A History of Alzheimer's Disease.* Bright Focus Foundation. 2014. http://www.brightfocus.org/alzheimers/about/understanding/history.html

- *Viral Vector.* Wikipedia. May 2014. http://en.wikipedia.org/wiki/Viral_vector

- *Medical Dictionary.* Farlex. http://medical-dictionary.thefreedictionary.com

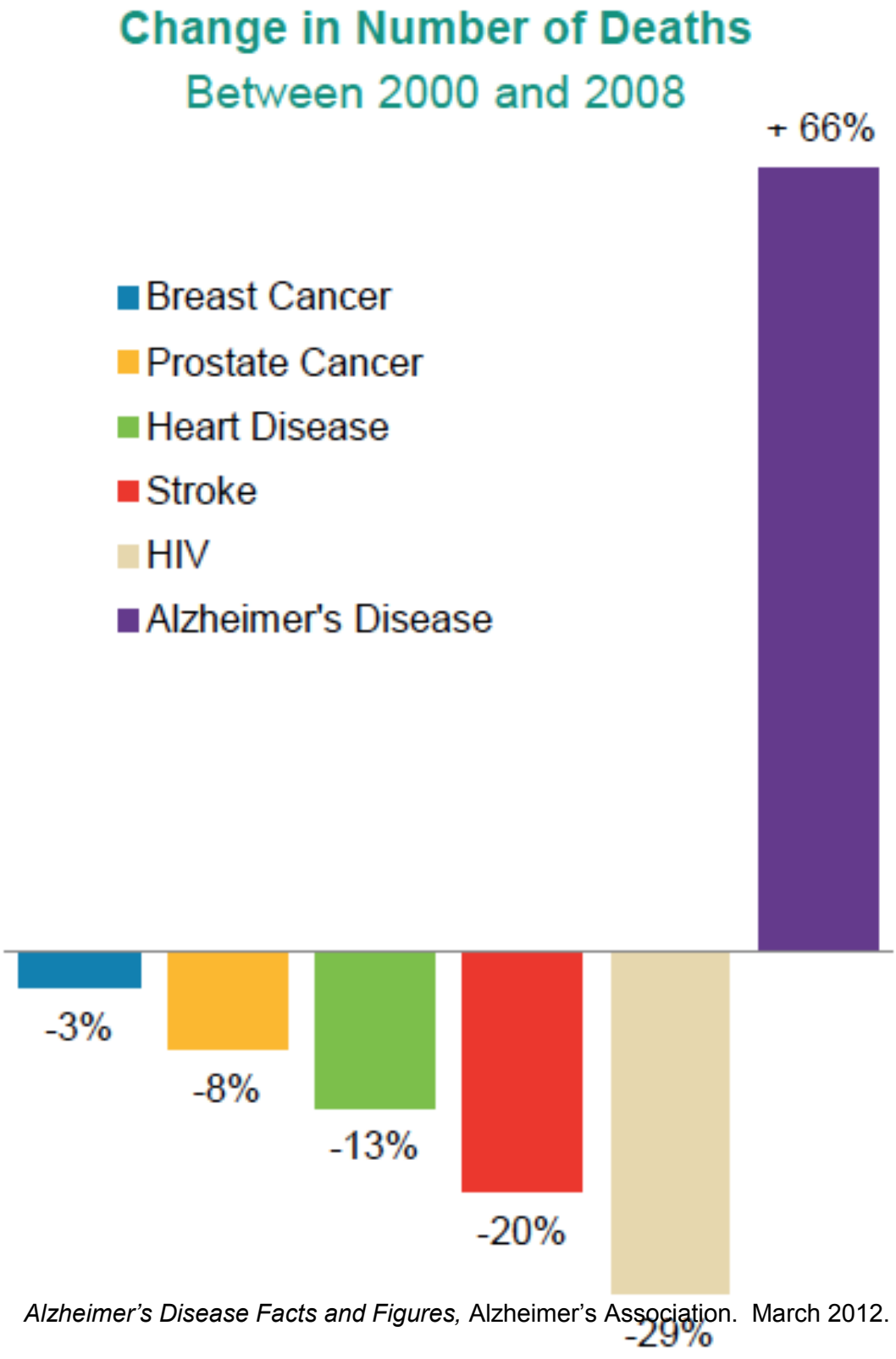
- *Viral Life Cycles in Cells.* Michigan State University. https://www.msu.edu/course/mmg/569/lifecycles.htm

- *How DNA Works*. Craig Freudenrich, Ph.D. 20 March 2007. http://science.howstuffworks.com/life/cellular-microscopic/dna.htm

- *Dictionary*. Dictionary.com. http://dictionary.reference.com

## Appendix

Graph.1




*Alzheimer's Disease Facts and Figures,* Alzheimer's Association. March 2012.

**4/11/2014, 18:00pm, Fundación Pascual Maragall. Interview to Joan Domingo Gispert:**

**Q:** *According to recent studies do we know what causes Alzheimer's?*

**A:** Not the ultimate cause. We only know that some weird things happen: Decades before the symptoms are seen there and pathological lesions in the brain that are characteristic of Alzheimer's. Approximately 5 years before the clinical symptoms start to appear there is already neurons dying in specific areas of the brain. The last stage before you start to see the symptoms, there are specific memory problems that often go unnoticed, but somehow we can maybe detect them with finer tests.
Because we do not know the cause of this disease, we try to characterize the evolution of the disease in the hope that through cohort studies, as the ALFA study done in the foundation, we will later be able to go to those people who develop the disease and look at the test that were done when they were all healthy, and try to see what the differences there are between those who develop the disease later, and those who never develop it.

**Q:** *How many research lines currently exist today?*

**A:** Endless. Global research lines are not centralized. Meaning that there is no investigation committee that tells you what to investigate.
Each person investigates what they want based on their own experiences, based on what they think they can discover or contribute with their experiences and resources. Each research group is independent from others.

In this field there is a unifying agent that is very important in the research, these agents are the pharmaceutical companies that do clinical trials. Meaning that on one hand there is academic research that is fundamentally distinct from public resources only attempting to gain knowledge about Alzheimer's. And on the other hand there is another type of research that is geared to develop drugs that cure or stop the disease and it's paid by pharmaceutical companies, but they also use academic resources in their studies.

Although there are endless research lines on the topic of drugs, some investigators are trying to find drugs that eliminate or clear the amyloid plaques in the brain. Other investigators are more oriented to prevent neurofibrillary tangles, which we do know that they appear when neurons are dying. There are other types of drugs that are more associated with the vascular hypothesis. Some say that Alzheimer's is a power failure of the neurons so they are investigating the part of the oxidization of the cells and they are trying to determine if the mitochondria works well. However, we haven't produced any drugs that change the course of the disease.

The problem is that will still don't know what agent causes this disease. So this indicates that maybe it's not an agent and it could be a sum of all kind of things that at the end participate in the disease.

**Q:** *My hypothesis is that the use of viral vectors in a treatment can be the cure of Alzheimer's. Do you agree?*

**A:** No, I don't agree. A viral vector can take something to a neuron that that neuron doesn't have, like in the case of Parkinson disease a viral vector can take dopamine to the neuron. But what happens in Alzheimer's is very different; there is nothing that the neuron is missing but there are leftovers. We have injuries that we cannot solve with a virus, nor can we regulate the production of amyloid plaques. The beta-amyloid plaques is a result of a protein that is located in the membrane of the neurons and without this protein the neurons will not survive. During the routine process of these proteins there are enzymes that "cut" these proteins. The problem in Alzheimer's is that one, there are too many "cuts" or two, once these proteins are divided they are not removed correctly so they pile up and aggregate to form the plaques. But we are not sure if this theory is correct. Furthermore this amyloid protein can go through our blood and end up in the brain. With all this, how would you be able to fix this with a virus?

**Q:** *We know that 1% of people with Alzheimer's have it because of genetic*

*reasons, couldn't we fix the genetic information of that cell to make it stop producing those amyloid plaques?*

**A:** No, a virus can only stop the expression of that gene. So instead of producing too much amyloid protein the neuron would stop synthesizing it leading to the death of them because it would not survive without it. However, there are drugs that regulate the production of this protein. There is one drug that inhibits it, called BACE1. But as you said this drug only works for that 1% of people.

**Q:** *Do you think that recently investigators have abandoned research lines related to proteins and now are focused on biomarkers? What research line are you in? Why?*

**A:** A big part of our investigation is about biomarkers. This is like this because we know that the disease starts in a silent way (20 years before the symptoms start to appear). Whereby we need biomarkers that describe the evolution of pathological processes that take place many years before. Why do we need them then? We need them to understand what is happening in this process and if we have any way to interrupt the pathological fall this could help us stop the progress of this disease. Detecting that preclinical stage of the disease gives us a window to test treatments on people that have these altered biomarkers, but that are not ill. And therefore try some kind of intervention to break the pathological fall so that person doesn't develop this illness.

Beta-amyloid research lines are not being abandoned but what is happening is that all the clinical trials that has been done until now are being done with ill people, with different grades of illness evolution, but always with the disease. The result of this is that they never work.
We have asked ourselves if some drugs are efficient if we apply them before the death of the neurons and the answer is that we have no clue. This is why we need to do clinical trials on people who aren't sick, or what is the same, design prevention studies not cure studies. This is the conceptual shift that has occurred in the last years regarding Alzheimer's.

**Q:** *Besides this process of detecting in advanced if someone will have Alzheimer's by means of biomarkers, is the foundation working on preventive treatments?*

**A:** We can't work with pharmaceutical drugs because only big companies do that. We collaborate with these companies when we are doing clinical trials so we can test the drugs, in this case, with people that still don't have the disease but they have positive biomarkers. It's still not official, but we are going to announce that we are participating en 3 different trials that are coordinated level 2 in Spain and level 1 in Europe. It will be made public in a few months that volunteers who are willing and meet the inclusion criteria will be able to participate. It's going to be a difficult trial because it's with people that are healthy and not a lot of people have those biomarkers.

**Q:** *What does the ALFA study consists of?*

**A:** They make a clinical history: the medications that they have taken, family history, etc. Then do some neuropsychological tests: test of pneumonia, calculation, etc. We take a blood sample to use genetic markers. In addition there are a number of questionnaires on life habits etc. MRI to see if there are structural alternatives in those brains. PET amyloid and function lumbar for biomarkers. With all these tests what we do is characterized from the point of view of clinical biomarkers and the genetics of these 2600 subjects and go periodically following every three years to see its evolution.

In principle, it is an indefinite project, which after some years unfortunately the volunteers might develop a type Alzheimer's dementia, others will have a tumor, others a different types of dementia, etc. So what we look into are those who end up having a dementia like Alzheimer's and then we initiate the data and try to find if there is any factor or factors that may explain why the disease develops so much and why others do not. We hope that these factors are identified and quantified. So we can give clear instructions to people like what would be correct lifestyles so they can prevent having this disease.

By now we are beginning to have evidence of what is good for the heart is also good for the brain. Today there is strong evidence of this. Just today a colleague sent me an article that says that people who do physical exercise between the ages of 45 and 65 have much more amyloid in the brain. The exercise becomes a protective factor, can't promise that exercise saves you from Alzheimer's, but if it doesn't then it clearly worsens their situation. If we can give life patterns to people we could lower the incidence of the disease. Also give us guidelines for research. Why is exercise so important for amyloid? Maybe because it requires more blood flow to eliminate toxicity of the brain. This is how science progresses, each time you solve a question, 10 more questions appear and you have to make 10 more experiments to answer these questions. Until one day, when we finally find something that will luckily work. I am convinced that this will happen, but I don't' know when. I am convinced that someday we will control Alzheimer's disease.

**Q:** *Do you know anyone that is specialized in viral vectors? Could you help me get an interview with him? If not, do you know someone that could give me more information about this treatment called Plasmapheresis done in the Grifols Labs with the ACE Foundation?*

**A:** I do not know anyone doing that test. But if you want my opinion it's an absolute outrage. Over 50 % of people have serious infections. Even if it was effective, you can't treat 100,000 patients in Catalonia that have Alzheimer's with a portacath and have 50,000 infected because of its fault. This can't be possible.
I was present in the last International Congress of Alzheimer's, where they presented the trial and there was no evidence that it is effective. This is a personal opinion of course! And I think that it's a type of study that we should not allow. I mean if you want to participate in a study that injects something directly in the brain and that may have the ability to change the course of their disease, sure I would say yes but due to fear of this type of treatment… It is the researcher who has to be fair to their volunteers and explain the risks of the study and the chances of success. There are other therapeutic ways that the same company Grifols is exploring that are much more promising. The vaccine, for example. This is a personal opinion. In science there are fashions, suddenly one thing becomes

fashionable, then as the years pass we have to ask ourselves in a critical way what has happened with these fashions.