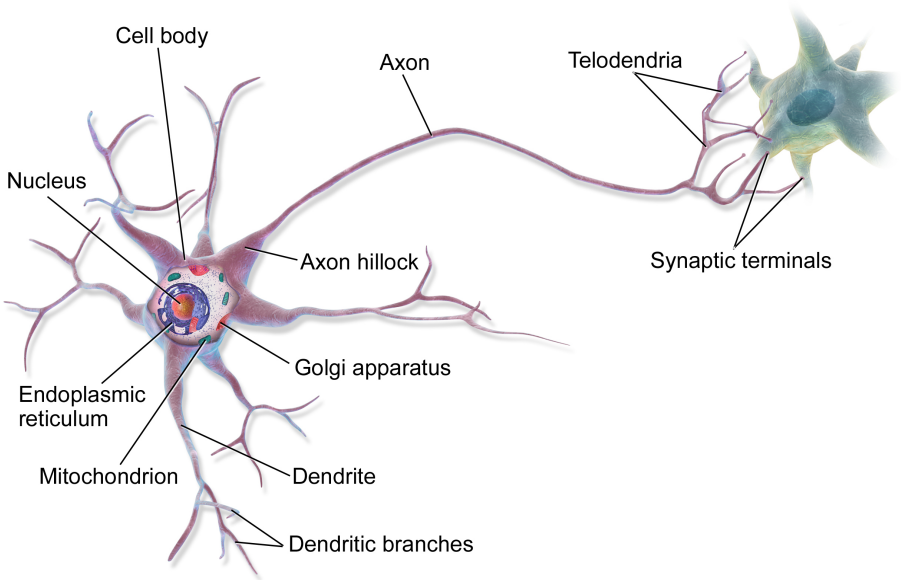
Timetable
Background research and topics
Testable question/Hypothesis
Research on hypothesis
Updated hypothesis
Science fair form
Research on updated hypothesis
Data/Conclusion
What next and applications

Timetable:

Due Date	Step	Status
Feb. 1-7	Better understanding of processes involved in the progression of AD like neuro-inflammation, misfolded proteins, amyloid-beta plaques, mitochondrial dysfunction, and other contributing elements of Alzheimer's disease as well as the types, stages, and complications.	
Feb. 8-14	Exploring inhibition of these processes from occurring at the onset.	
Feb. 15-21	Studying clinical trials that are based on the concept of combination therapy involving two or more drugs that target two different causes/processes.	
Feb. 22-28	Investigating the mechanism of the drugs that are currently being tested.	
Mar. 1-7	Deriving conclusions from the literature reviews. Searching for additional supportive	

	information e.g.: facts/ diagnostics/ treatments using other strategies.	
Mar. 8-14	Proposing a possible combination of drugs that could be used and identifying possible areas that require further research.	
Mar. 14-19	Upload research onto website/Create presentations and video.	

Background Research/ How did I choose this topic:
https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease
https://en.wikipedia.org/wiki/Neuron#/media/File:Blausen_0657_MultipolarNeuron.png- Image 1
https://www.drugabuse.gov/sites/default/files/images/colorbox/neurotrans_graphic3.jpg Image 2
https://d3i71xaburhd42.cloudfront.net/a91101093ef41290be2dd86c8780ef7ee2c004de/2-Figure1-1.png Image 3
https://wustljcubes.files.wordpress.com/2015/06/brain-scans-544x360.jpg Image 4
https://www.pnnl.gov/sites/default/files/styles/hero_1600x1200/public/media/image/CellularHealth_HERO.jpg?itok=LkozCNbv Image 5

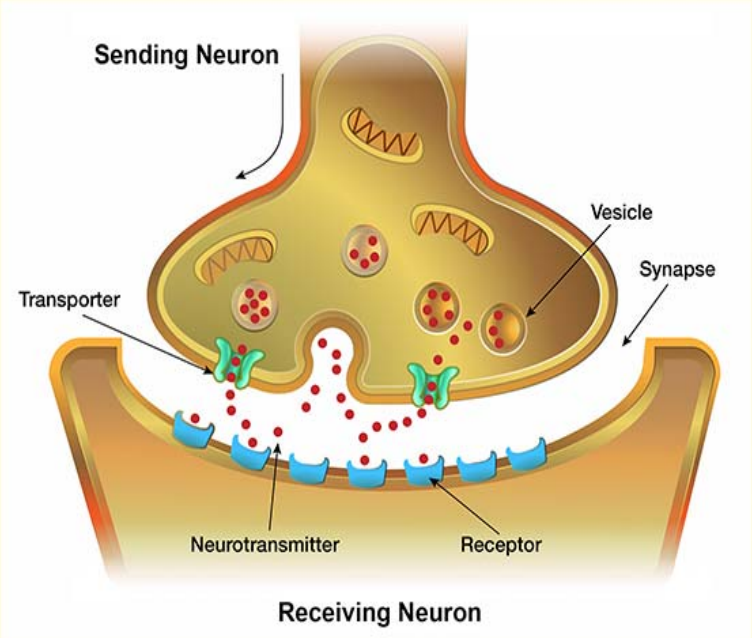


Alzheimer's dementia disrupts communication among neurons, resulting in loss of function and cell death. Most neurons have three basic parts: a cell body, multiple dendrites, and an axon.

- The cell body contains the nucleus, which houses the genetic blueprint that directs

and regulates the cell's activities.

- Dendrites are branch-like structures that extend from the cell body and collect information from other neurons.
- The axon is a cable-like structure at the end of the cell body opposite the dendrites and transmits messages to other neurons.
- Dendrite collects information while the axon sends the messages

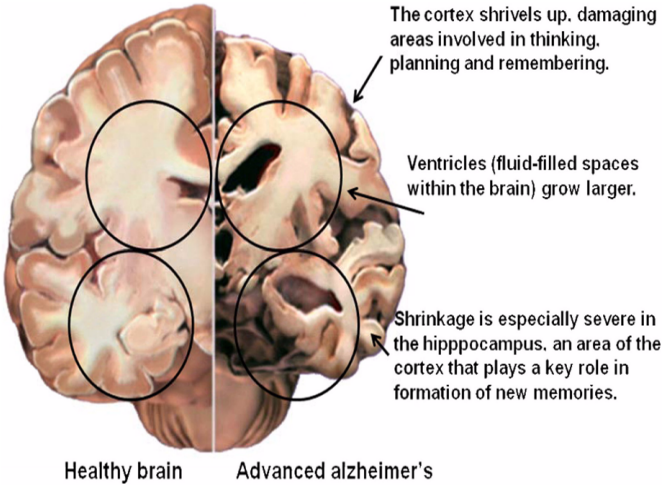


The function and survival of neurons depend on several key biological processes:

- **Communication**: Neurons are constantly in touch with neighbouring brain cells.
    - When a neuron receives signals from other neurons, it generates an electrical charge that travels down the length of its axon and releases neurotransmitter chemicals across a tiny gap, called a synapse.
        - (Neurotransmitters are chemical messengers that transmit a message from a nerve cell across the synapse to a target cell. The target can be another nerve cell, or a muscle cell, or a gland cell. They are chemicals made by the nerve cell specifically to transmit the message. Neurotransmitters are released from synaptic vesicles in synapses into the synaptic cleft, where they are received by neurotransmitter receptors on the target cell.)
    - Each neurotransmitter molecule then binds to specific receptor sites on a dendrite of a nearby neuron.
    - This process triggers chemical or electrical signals that either stimulate or inhibit activity in the neuron receiving the signal.
    - Scientists estimate that in the brain's communications network, one neuron may have as many as 7,000 synaptic connections with other neurons.
- **Metabolism:** Metabolism—the breaking down of chemicals and nutrients within a cell—is critical to healthy cell function and survival.

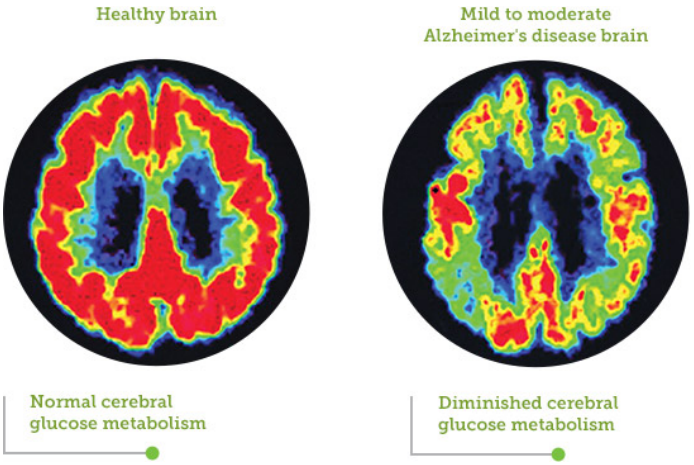
- To perform this function, cells require energy in the form of oxygen and glucose, which are supplied by blood circulating through the brain.
- The brain has one of the richest blood supplies of any organ and consumes up to 20 percent of the energy used by the human body—more than any other organ.

- **Repair, remodeling, and regeneration**: Neurons have evolved to live a long time—more than 100 years in humans.
  - neurons must constantly maintain and repair themselves
  - Neurons also continuously adjust, or "remodel," their synaptic connections depending on how much stimulation they receive from other neurons
    - they may strengthen or weaken synaptic connections, or even break down connections with one group of neurons and build new connections with a different group
  - Adult brains may even generate new neurons—a process called neurogenesis
    - Remodeling of synaptic connections and neurogenesis are important for learning, memory, and possibly brain repair.

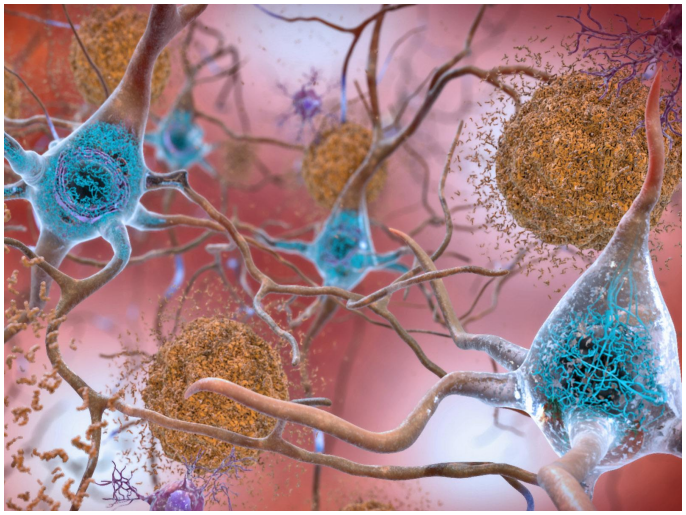


So, How does Alzheimers affect the brain?

- A healthy brain shrinks in aging but doesn't lose neurons. In Alzheimer's disease damage is widespread as many neurons stop functioning, lose connections with other neurons, and die.
- disrupts processes vital to neurons and their networks, including communication, metabolism, and repair.
- At first, Alzheimer's disease typically destroys neurons and their connections in parts of the brain involved in memory, including the entorhinal cortex and hippocampus.
- Then affects areas in the cerebral cortex responsible for language, reasoning, and social behavior.
- Later many other areas of the brain are damaged



- Over time, a person with Alzheimer's gradually loses his or her ability to live and function independently. Ultimately, the disease is fatal.
- Neurofibrillary tangles are abnormal accumulations of a protein called tau that collect inside neurons. Healthy neurons, in part, are supported internally by structures called microtubules, which help guide nutrients and molecules from the cell body to the axon and dendrites. In healthy neurons, tau normally binds to and stabilizes microtubules. In Alzheimer's disease, however, abnormal chemical changes cause tau to detach from microtubules and stick to other tau molecules, forming threads that eventually join to form tangles inside neurons. These tangles block the neuron's transport system, which harms the synaptic communication between neurons.



- Emerging evidence suggests that Alzheimer's-related brain changes may result from a complex interplay among abnormal tau and beta-amyloid proteins and several other factors. It appears that abnormal tau accumulates in specific brain regions involved in memory. Beta-amyloid clumps into plaques between neurons. As the level of beta-amyloid reaches a tipping point, there is a rapid spread of tau throughout the brain.
-

There are two types of protein responsible for this disease: Beta-Amyloid and Tau.

- Become toxic to the brain
- Tau accumulates as tangles inside neurons and Beta-amyloid clumps as plaques blocking passageways between neurons.
- As Beta-amyloid plaques build up, tau tangles increase in the brain, blocking the pathways to vital processes in the brain such as lack of blood from the vascular system causing the lack of glucose needed to perform metabolism.
- Chronic Inflammation sets in as microglial cells are unable to clear the debris and help keep neurons healthy.
    - Microglia and astrocytes are the janitor cells in your brain responsible to clear away debris and keep things healthy

- This causes the neurons to suddenly lose their communication and they start to die
- As that happens, the brain begins shrinking starting with the Hippocampus: Important to learning and memory.
    - Slowly, people begin to experience memory loss, impaired decision making as well as language problems.

Where does amyloid-beta come from? What is it? How does it contribute to Alzheimer's?

The amyloid-beta comes from this larger protein called Amyloid-beta Precursor Protein (APP). The function of this protein is relatively unknown to us because of its layers of domains making it difficult to study as one piece.

- APP plays roles both as an intact membrane protein and when broken into pieces.
    - The intact protein is a receptor protein that sends signals through the G-protein system.
        - The G-proteins (**guanine nucleotide-binding proteins)** are a family of proteins that act as molecular switches inside cells and are involved in transmitting signals from a variety of stimuli outside a cell to its interior.
        - GTP is involved in energy transfer within the cell.
        - It is also involved in genetic transfer and mitochondrial function along with many other processes.
        - G proteins belong to the larger group of enzymes called GTPases.
        - G proteins are important signal transducing molecules in cells**.**
    - APP also binds to many structural molecules outside cells, such as heparin and laminin, so it may play a role in cell adhesion.
    - APP is also broken into several functional fragments by a set of dedicated proteases, termed secretases.
        - A protease is an enzyme which breaks down proteins and peptides.
        - Secretases are enzymes that "snip" pieces off a longer protein that is embedded in the cell membrane.
        - Among other roles in the cell, secretases act on the Amyloid Precursor Protein (APP) to cleave the protein into three fragments.

The brain has APP which is cleaved by 3 secretases: alpha, beta, gamma. In a normal brain, the alpha secretase acts on APP and cleaves of sAPPa (secreted APP alpha).

What is left is an 83 amino acid long membrane-bound C-terminal fragment called CTF83. In a brain with Alzheimers's, an enzyme called beta-secretase acts on APP and cleaves it into sAPPb (secreted APP beta). What is left is a 99 amino acid long membrane-bound c-terminal fragment called CTF99. In normal signaling, CTF83 is further cleaved by the gamma-secretase complex made up of PSEN1, Pen-2, APH-1, GSAP, and NCT. Cleavage of CTF83 leads to the generation of APP intracellular domain (AICD fragment). AICD fragment translocates to the nucleus where it affects the transcriptional regulation of several proteins, and drives neuroprotective pathways. It is also important to know that sAPP alpha gets secreted from the neurons and drives normal synaptic signaling leading to synaptic plasticity, learning and memory, neuronal survival, and emotional behaviours. In AD, gamma-secretase complex is also assembled but cleaves CTF99 fragment into an AICD fragment and an abeta 40/42 peptide. AICD is again translocated to the nucleus where it affects transcriptional regulation of several proteins and drives neuroprotective pathways. The Abeta 40/42 peptide however is involved in downstream pathways related to AD.

https://youtu.be/brq0SLXbLfA

Tau tangles:

Tau is a protein that helps stabilize the internal skeleton of nerve cells (neurons) in the brain. This internal skeleton has a tube-like shape through which nutrients and other essential substances travel to reach different parts of the neuron. In Alzheimer's disease, an abnormal form of tau builds up and causes the internal skeleton to fall apart.

Emerging evidence suggests that Alzheimer's-related brain changes may result from a complex interaction among abnormal tau and beta-amyloid proteins and several other factors. It appears that abnormal tau accumulates in specific brain regions involved in memory. As the amount of beta-amyloid in the brain increases, a tipping point is reached that causes abnormal tau to spread throughout the brain. Until recently, tau and beta-amyloid levels in a person with dementia could only be measured after the person had died. Now, however, tau and beta-amyloid levels can be measured in living individuals by analyzing samples of cerebrospinal fluid (CSF), the fluid surrounding the brain, and by using positron emission tomography (PET) scans and special dyes to show tau tangles and beta-amyloid plaques in the brain. Scientists are beginning to use CSF analysis and PET scans to study how tau and beta-amyloid may interact to speed

the brain changes that ultimately result in memory loss and other symptoms of Alzheimer's dementia.

Tau accumulation has been shown to promote brain cell damage and death in Alzheimer's and other dementias, including frontotemporal dementia, but the exact processes that lead to this toxicity are unclear. Some studies suggest stress in the brain cell's endoplasmic reticulum (ER), the part of the cell where proteins are produced, may play a role in the toxic effect of tau tangles and abnormal tau. Results of these studies may clarify how tau toxicity develops in the brain and promotes brain cell damage in Alzheimer's. The results may also suggest new avenues to investigate for therapies that may prevent or slow the brain changes of Alzheimer's and other dementias.

Researchers are investigating ways to prevent tau protein from forming into tangles, which ultimately destroys the neuron. One potential therapy in clinical trials that targets tau protein is AADvac1. AADvac1 is a vaccine that stimulates the body's immune system to attack the abnormal form of tau protein that causes the internal skeleton of neurons to fall apart. If successful, it has the potential to help stop the progression of Alzheimer's disease.

Stages and Symptoms of Alzheimer's Disease:

https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet#:~:text=Alois%20Alzheimer.,language%20problems%2C%20and%20unpredictable%20behavior.

https://www.alz.org/alzheimers-dementia/what-is-alzheimers

Stages: There are 3 overlapping phases; early, middle and late (sometimes referred to as mild, moderate and severe in a medical context)

- On average, a person with Alzheimer's lives four to eight years after diagnosis, but can live as long as 20 years, depending on other factors.
- Changes in the brain related to Alzheimer's begin years before any signs of the disease.

- This time period, which can last for years, is referred to as preclinical Alzheimer's disease.
- Dementia is a general term to describe the symptoms of mental decline that accompany Alzheimer’s and other brain diseases.

Early-stage Alzheimer's (mild)

- person may function independently
- He or she may still drive, work and be part of social activities
- Despite this, the person may feel as if he or she is having memory lapses, such as forgetting familiar words or the location of everyday objects.
- Symptoms may not be widely apparent at this stage, but family and close friends may take notice and a doctor would be able to identify symptoms using certain diagnostic tools.
- Common difficulties include:
    - Coming up with the right word or name.
    - Remembering names when introduced to new people.
    - Having difficulty performing tasks in social or work settings.
    - Forgetting material that was just read.
    - Losing or misplacing a valuable object.
    - Experiencing increased trouble with planning or organizing.

Middle-stage Alzheimer's (moderate)

- Middle-stage Alzheimer's is typically the longest stage and can last for many years.
- During the middle stage of Alzheimer’s, the dementia symptoms are more pronounced.
- the person may confuse words, get frustrated or angry, and act in unexpected ways, such as refusing to bathe
- Damage to nerve cells in the brain can also make it difficult for the person to express thoughts and perform routine tasks without assistance.

Symptoms, which vary from person to person, may include:

- Being forgetful of events or personal history.
- Feeling moody or withdrawn, in socially or mentally challenging situations.
- Being unable to recall information about themselves like address or telephone number, and the high school or college they attended.
- Experiencing confusion about where they are or what day it is.
- Requiring help choosing proper clothing for the season or the occasion.

- Having trouble controlling their bladder and bowels.
- Experiencing changes in sleep patterns, such as sleeping during the day and becoming restless at night.
- Showing an increased tendency to wander and become lost.
- Demonstrating personality and behavioral changes, including suspiciousness and delusions or compulsive, repetitive behavior like hand-wringing or tissue shredding.

Late-stage Alzheimer's (severe)

- In the final stage of the disease, dementia symptoms are severe.
- Individuals lose the ability to respond to their environment, to carry on a conversation and, eventually, to control movement.
- They may still say words or phrases, but communicating pain becomes difficult.
- At this stage, individuals may:
    - Require around-the-clock assistance with daily personal care.
    - Lose awareness of recent experiences as well as of their surroundings.
    - Experience changes in physical abilities, including walking, sitting and, eventually, swallowing
    - Have difficulty communicating.
    - Become vulnerable to infections, especially pneumonia.

Understanding Alzheimer’s Better:

- Alzheimer's is the most common cause of dementia,
- Alzheimer's disease accounts for 60-80% of dementia cases.
- Alzheimer's is not a normal part of aging.
- Approximately 200,000 Americans under the age of 65 have younger-onset Alzheimer’s disease (also known as early-onset Alzheimer’s).
- Alzheimer's is a progressive disease, where dementia symptoms gradually worsen over a number of years
- Alzheimer's is the sixth leading cause of death in the United States.
- Alzheimer's has no current cure, but treatments for symptoms are available
- Although current Alzheimer's treatments cannot stop Alzheimer's from progressing, they can temporarily slow the worsening of dementia symptoms
- The most common early symptom of Alzheimer's is difficulty remembering newly learned information because Alzheimer's changes typically begin in the part of the brain that affects learning.

- Microscopic changes in the brain begin long before the first signs of memory loss.

The role of plaques and tangles

- Two abnormal structures called plaques and tangles are prime suspects in damaging and killing nerve cells.
    - Plaques are deposits of a protein fragment called beta-amyloid that build up in the spaces between nerve cells.
    - Tangles are twisted fibers of another protein called tau that build up inside cells.
- Though autopsy studies show that most people develop some plaques and tangles as they age, those with Alzheimer's tend to develop far more and in a predictable pattern, beginning in the areas important for memory before spreading to other regions.
- they somehow play a critical role in blocking communication among nerve cells and disrupting processes that cells need to survive.
- It's the destruction and death of nerve cells that causes memory failure, personality changes, problems carrying out daily activities

Origin of disease

- In 1906, German physician Dr. Alois Alzheimer first described "a peculiar disease" — one of profound memory loss and microscopic brain changes — a disease we now know as Alzheimer's.
- Alzheimer's disease is named after Dr. Alois Alzheimer. In 1906, Dr. Alzheimer noticed changes in the brain tissue of a woman who had died of an unusual mental illness.
- Her symptoms included memory loss, language problems, and unpredictable behavior.
- After she died, he examined her brain and found many abnormal clumps (now called amyloid plaques) and tangled bundles of fibers (now called neurofibrillary, or tau, tangles).

Alzheimer's Genetics:

- Most people with Alzheimer's have the late-onset form of the disease, in which symptoms become apparent in their mid-60s.
- having one form of the apolipoprotein E (APOE) gene does increase a person's risk. This gene has several forms. One of them, APOE ε4, increases a person's risk of developing the disease and is also associated with an earlier age of disease onset.

- carrying the APOE ε4 form of the gene does not mean that a person will definitely develop Alzheimer's disease, and some people with no APOE ε4 may also develop the disease.
- Early-onset Alzheimer's disease occurs between a person's 30s and mid-60s and represents less than 10 percent of all people with Alzheimer's
- Most people with Down syndrome develop Alzheimer's. This may be because people with Down syndrome have an extra copy of chromosome 21, which contains the gene that generates harmful amyloid.

How Is Alzheimer's Disease Diagnosed?

- Ask the person and a family member or friend questions about overall health, use of prescription and over-the-counter medicines, diet, past medical problems, ability to carry out daily activities, and changes in behavior and personality
- Conduct tests of memory, problem solving, attention, counting, and language
- Carry out standard medical tests, such as blood and urine tests, to identify other possible causes of the problem
- Perform brain scans, such as computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET), to rule out other possible causes for symptoms
- Alzheimer's disease can be *definitely* diagnosed only after death, by linking clinical measures with an examination of brain tissue in an autopsy.

Medications to Maintain Mental Function in Alzheimer's Disease

- Donepezil (Aricept®), rivastigmine (Exelon®), and galantamine (Razadyne®) are used to treat mild to moderate Alzheimer's (donepezil can be used for severe Alzheimer's as well).
- Memantine (Namenda®), the Exelon® patch, and Namzaric® (a combination of memantine and donepezil) are used to treat moderate to severe Alzheimer's.
- These drugs work by regulating neurotransmitters, the chemicals that transmit messages between neurons
- They may help reduce symptoms and help with certain behavioral problems.
- However, these drugs don't change the underlying disease process.
- They are effective for some but not all people, and may help only for a limited time.

Definition of Terms:

Amino acids - Amino acids are organic molecules that are used to make proteins. They consist of elements such as carbon, hydrogen, oxygen and nitrogen.
Proteins - They are a chain of amino acids.

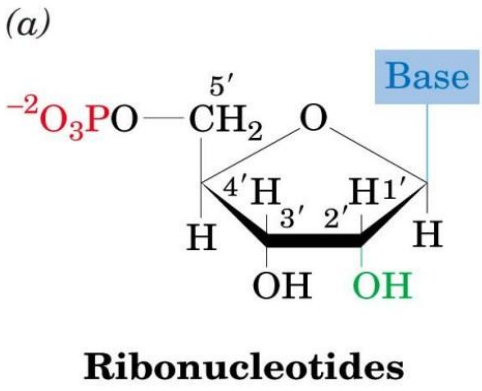
Proteins are made inside the cell. This process is called protein synthesis. The process starts in the cell's DNA which rests in the cell's nucleus. The DNA holds instructions for how to make the protein which is possible with two stages: Transcription and translation.

Transcription: The cell makes a copy/transcript of the DNA (Deoxyribonucleic Acid). This is called RNA because it is made up of ribonucleic acid. The name is derived from the sugar which is bound to the base. For RNA it is Ribose (that is why it is called ribonucleic acid) and for DNA it is Deoxyribose (hence the name deoxynucleic acid). The deoxyribose is missing an OH-group at position 2 of the sugar ring, the name literally means "without oxygen".

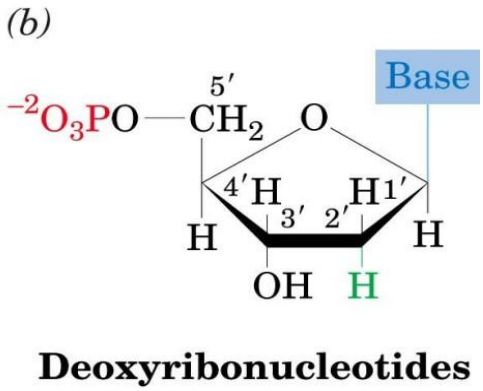
The next step is translation

Translation: The RNA is converted/translated into a sequence of amino acids that make up a protein. This process takes place in the ribosome.

1. The RNA moves to the ribosome. This is called the messenger RNA (mRNA)
2. mRNA attaches itself to the ribosome
3. The ribosome figures out when to start on the mRNA by finding the three letter “begin” sequence called a codon
4. The ribosome then moves down the strand of mRNA. The ribosome builds a string of amino acids based on the codes in the mRNA.



Ribonucleotides



Deoxyribonucleotides

5. When the ribosome sees the “stop” code, it ends the translation and the protein is complete.

There are many types of proteins:

1. Structural: provide structure in our body.
2. Defensive: help fight disease, create antibodies.
3. Transport: carry essential nutrients, like hemoglobin carried blood and oxygen

4. Catalysts: like enzymes, assist in chemical reactions. Help break up and digest food so that the energy can be used as energy. Accelerate chemical reactions. Enzymes are what break up APP.

If there are less of these secretases, will it reduce the AB plaques? Will it affect the brain? How will it? Is there a way to prevent the formation of these plaques. Is it possible for the alpha-secretase to cleave instead of beta and gamma. If these processes are inhibited, can the plaques not form? How can they be inhibited?

Formulation of Question #1: Will inhibiting/preventing Amyloid-beta production be an effective treatment for Alzheimer's Dementia?

Hypothesis #1: Identifying the process in the brain through which beta-amyloid plaques form and inhibiting those effects from happening through inhibitors will play a role in reversing the effects of Alzheimer's Dementia by reducing the chance of AB plaques in the brain.

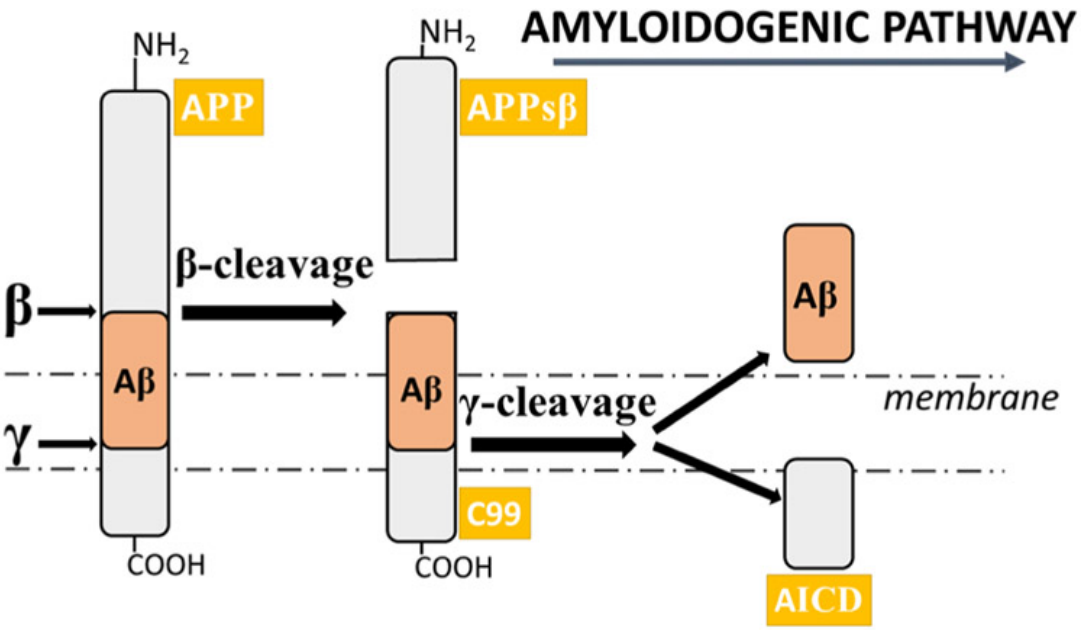
BACE inhibitors as an effective treatment for alzheimers:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7330928/

Human genetic and clinical studies have understood and indicated that accumulation of amyloid-B (AB) peptides is a possible culprit in the pathogenesis of AD. AB is generated through proteolytic cleavage of the Amyloid Precursor Protein by the b-site APP cleaving enzyme (BACE-1) (memapsin 2, β-secretase, Asp 2 protease) and γ-secretase.

Memapson 1 and 2 were originally found in the expressed sequence tag (EST) These proteases are unique among aspartic proteases in that they are membrane-anchored. Detailed enzymic and cellular studies suggest that M2 fits all of the criteria of *β*-secretase. Like M2, APP is a type I integral transmembrane protein and is known to be processed *in vivo* at three sites. These three sites are a-secretase site, b-secretase site, and y-secretase site. The evidence suggests that cleavage at the α-secretase site by a membrane-associated metalloprotease is a physiological event (a response to stimuli). This site is located in APP 12 residues away from the luminal surface of the plasma membrane. The b-secretase site is located 28 residues away from the plasma membrane's luminal surface. The y-secretase is located in the transmembrane region. Cleavage of the b-secretase site and the y-secretase sight results in a 40- 42 residue amyloid-b peptide (AB) whose accumulation results in amyloid-beta plaques in the brain that cause AD.

- Mice deficient in BACE1 show abrogated production of Aβ. Therefore, pharmacological inhibition of BACE1 is being intensively pursued as a therapeutic approach to treat AD patients.
- As illustrated by the amyloid hypothesis amyloid deposits likely occur prior to the formation of neurofibrillary tangles
- It has been reported that genetic mutations in the tau-coding gene can pathologically induce tau aggregation, suggesting that the formation of neurofibrillary tangles can be independent of toxic Aβ components.
- neurofibrillary tangles appear to be more associated with neuronal and synaptic losses, which are two typical neuropathologies leading to memory impairment, confusion, personality changes, and cognitive decline over time in AD.
    - Hence, either Aβ or tau oligomers can damage the neuronal communication via damaging synaptic connections.

The prevention of synaptic loss or preservation of synaptic connections should be a viable strategy to stop Alzheimer's patients from developing memory problems.



While aggregate AB is a major cause of AD pathogenesis, functions relating to apolipoprotein E4 (ApoE4), tau, a-synuclein, and TDP-43 are likely comorbidity factors.

- Other pathological factors such as: inflammatory response, ion homeostasis disruption, oxidative stress, and decreased levels of neurotransmitters have also been identified and are considered to be sequential events of Aβ aggregation.
    - **Growth of amyloid plaques, both in size and density, is age-dependent; this is consistent with the fact that aging is the most important non-genetic risk factor for AD. p**
- Aβ oligomerizes and aggregates into small clusters or a nidus outside neurons, prior to the formation of dense core senile plaques.
- Activation of microglia has been shown to migrate towards such growing Aβ plaques , where reactive astrocytes can also be found concurrently.

1. It has been shown that deletion of BACE-1 (beta-site amyloid precursor protein cleaving enzyme 1) abrogates AB production and betters cognitive/behavioral deficiencies, as observed in transgenic mice overexpressing human APP with familial AD mutations, indicating that inhibition of BACE1 has a direct effect.
2. “A rare human mutation at the BACE1 cleavage site of APP [A673T] results in a 40% decrease in Aβ production in vitro, a significantly reduced propensity for Aβ to aggregate, a five- to seven-fold reduced risk of developing AD, and greater resilience to cognitive dysfunction in elderly individuals, implying that BACE1 cleavage alone appears to be beneficial in the human brain.”
3. inhibition of BACE1 directly reduces Aβ-mediated impairments in synaptic transmission.
4. Deletion of BACE1 in mice appears to have a minor impact on mouse growth or overall functions, perhaps related to the fact that most BACE1 substrates are also shed by α-secretase.
5. Direct inhibition of γ-secretase, another strategy to reduce Aβ generation, is now recognized to be more challenging due to the indispensable physiological roles of γ-secretase, leading investigators and many companies to focus on BACE1 inhibitors, which act upstream of γ-secretase in Aβ generation.

Thus, BACE1 is recognized as a better-positioned target for treating AD patients.

What really is BACE-1? How can it be inhibited?

- The crystal structure of BACE1 reveals that the proteolytic pocket of BACE1 is relatively large and less hydrophobic, and can accommodate up to 11 residues.
    - Renders it more challenging for the development of small molecule inhibitors using the high throughput screening approach.
        - **“High throughput** screening (HTS) is the use of automated equipment to rapidly test thousands to millions of samples for

biological activity at the model organism, cellular, pathway, or molecular level." https://www.sciencedirect.com/topics/medicine-and-dentistry/high-throughput-screening

- The practical BACE1 inhibitors should be small in size and cross the blood–brain barrier easily because BACE1 is richly expressed in the brain, mostly in neurons, and is readily detected in presynaptic hippocampal mossy fiber terminals.
    - By crossing the blood–brain barrier, BACE-1 inhibitory drugs will be able to reduce AB levels in neurons and in the brain overall

A few inhibitory drugs were developed that targeted BACE-1.

1. Verubecestat: https://www.alzforum.org/therapeutics/verubecestat
    - Is the first small-molecular BACE1 inhibitor with oral availability and blood–brain barrier permeability
    - Verubecestat forms interactions between the amidine moiety of verubecestat and the BACE1 catalytic dyad through hydrogen bonding.
    - Long-term treatment of verubecestat in animals can strongly reduce Aβ40, Aβ42, and soluble peptide APPβ Soluble Amyloid Precursor Protein (sAPPβ) in cerebrospinal fluid (CSF) and the brain.
    - In the initial phase I trial that tested single doses up to 450 mg and multiple doses from 12 to 150 mg/day, verubecestat was well-tolerated and safe while a reduction of the CSF Aβ concentration in 32 participants reached ~ 90%. Another trial further confirmed tolerability to single and multiple doses.
    - The phase II/III EPOCH trials focused on evaluation of conventional cognitive and functional outcomes.
    - Among the 1958 individuals recruited with moderate or mild AD, 80% were Caucasian and 63% had at least one ApoE4 allele; their Mini-Mental State Examination (MMSE) scores were between 15 and 28. They received either 12 or 40 mg of study drug or placebo for 18 months.
    - Most participants either had taken or currently took a cholinesterase inhibitor and/or memantine.
        - Cholinesterase inhibitors aim to increase communication between the nerve cells to try to improve the symptoms of Alzheimer's. These drugs have been approved for use in mild to moderate Alzheimer's disease.
        - Memantine is used to treat the symptoms of Alzheimer's disease (AD; a brain disease that slowly destroys the memory and the ability to think, learn, communicate and handle daily activities).

Memantine is in a class of medications called NMDA receptor antagonists. It works by decreasing abnormal activity in the brain.

- Although a dramatic reduction of Aβ40, Aβ42, and sAPPβ in CSF of up to 80% was detected and a small reduction in plaque load was confirmed by amyloid PET in participants taking the drug, the clinical trial was terminated in February 2018, with verubecestat exhibiting no improvement in cognitive function in AD patients cited as the reason.
- The decline on the cognitive scale was at the same rate in both groups.
- No impact of this drug on either total Tau (T-Tau) and phosphorylated Tau (P-Tau) levels in the treated group.
- Rashes were almost twice as common in the verubecestat group than with placebo.
- Changes in hair color were frequent and this side effect is related to the inhibition of BACE2 for its control of hair pigmentation
- The administration of verubecestat in people with prodromal AD (APECS trial) resulted in a worsening of cognitive symptoms
- The inefficiency of the drug in improving cognitive function becomes complicated, perhaps being concealed by a secondary neuropsychiatric effect.

The results of this drug trial suggest that BACE1 inhibitors need to be given several years before the onset of AD symptoms.

2. Lanabecestat: https://www.alzforum.org/therapeutics/azd3293
    - Lanabecestat, a small-molecule, orally administered BACE1 inhibitor developed by AstraZeneca, was first extensively tested in primary cortical neurons, mice, guinea pigs, and dogs prior to clinical trials
    - The drug has a slow off-rate (estimated half-life of 9 h for BACE1) , which may result in a prolonged reduction of Aβ.
    - The phase I study, begun in 2014 and trial results demonstrated excellent safety, tolerability, and metabolic profiles in elderly healthy volunteers and in AD patients with mild cognitive impairment
    - A separate phase I study in Japan also revealed excellent pharmacokinetics for the drug.
    - Like Merck's verubecestat, lanabecestat also strongly decreased the CSF Aβ level in the treated group.
    - Phase II/III clinical trials recruited over 1400 participants and were planned to last up to 54 months with variable doses; they attempted to measure efficacy and safety in humans by analyzing results such as amyloid PET scans, CSF Aβ levels, and CSF amyloid inclusion.

    - The primary functional outcome from these rigorous trials was anticipated to be improved patient cognition in addition to decreasing CSF levels of Aβ40, total tau, and phosphorylated tau.
- Compound also causes depigmentation in the epidermis and hair
- The clinical development program of this compound largely skipped Phase 2. Instead of running a medium-size Phase 2 followed by separate, larger confirmatory Phase 3 trials, the sponsors opted for a large, pivotal Phase 2/3 trial called AMARANTH.
- This trial compared AZD3293 to placebo given for two years in 2,202 patients who met NIA-AA criteria for MCI due to AD or mild AD.
- Each participant or his or her partner was required to report worsening in the past six months, and the participant's MMSE had to be above 21 at screening.
- To ascertain that they had brain amyloid accumulation, participants either underwent an amyloid PET scan or a lumbar puncture and continued in respective sub-studies monitoring those markers for treatment response.
- This international trial began enrolling in December 2014, and was set to run until 2019.
- In July 2016, a second Phase 3 trial started up. Called DAYBREAK-ALZ and conducted at 251 locations worldwide, it enrolled 1,899 patients with mild AD dementia as defined by an NIA-AA diagnosis of probable AD with a biomarker evidence of brain amyloid and an MMSE of 10 to 26.
- This four-arm trial compared two once-daily doses given for three years to two groups who start out on placebo for 18 months and then switch to either the low or high dose for the second half of the trial.
- This trial was set to run until 2021.
- On June 12, 2018, AMARANTH and DAYBREAK-ALZ were discontinued due to lack of efficacy determined at an interim futility analysis
- In AMARANTH, the larger trial, neither 20 nor 50 mg per day moved any of the primary or secondary outcome measures; the placebo group declined at the expected rate.
- The most common serious side effects were psychiatric, 2 kg weight loss, and hair discoloration.
- Likewise, DAYBREAK revealed no treatment benefit on any endpoint, with similar side effects.
- Lanabecestat groups showed worsening on the RBANS Total Score, Immediate Memory, Visuospatial/Constructional indexes, and Digit Symbol Coding, but improvement in verbal fluency tests.
- Lanabecestat reduced blood Aβ40 and Aβ42 levels by 70 to 80 percent in both trials.

- CSF Aβ, measured in AMARANTH, dropped by 50 and 73 percent at the low and high doses, respectively.
- Lanabecestat dose-dependently reduced brain amyloid on florbetapir-PET imaging.

Similar to that of verubecestat, the lesson learnt from this announcement is that the appearance of even mild symptoms may be too late in the disease continuum for a BACE1 inhibitor to be efficacious.

3. Atabecestat
    - has been developed by Shionogi as a brain-penetrable small-molecular BACE1 inhibitor.
    - In collaboration with Janssen, this orally available BACE1 inhibitor has entered a phase I trial
    - Daily administration of atabecestat 5–150 mg in healthy elderly and young participants for up to 14 days showed significant and consistent reduction of Aβ (up to 90% in the 90 mg cohort) in both plasma and CSF
    - Another phase I trial in Japan aimed to evaluate a 1-month course of 10 or 50 mg in 18 patients with brain Aβ deposits and low CSF Aβ42 levels
        - These participants were categorized as “asymptomatic at risk of AD” as they exhibited an earlier stage of AD pathophysiology than pre-dementia or prodromal AD.
    - A recently published study of two similarly designed phase I trials including Caucasian and Japanese patients administered atabecestat 10 and 50 mg for 4 weeks demonstrated mean CSF Aβ1-40 reductions of 67% and up to 90%, respectively
    - Although minor adverse effects such as headache and back pain were noted in the phase I trial, it was deemed safe enough to advance to phase II trials.
    - a multicenter phase II trial, recruited 114 pre-dementia individuals to determine the tolerability and long-term safety of atabecestat, including double-blind treatment for 6 months.
    - Atabecestat was found to successfully reduce both plasma and CSF levels of Aβ1-37, Aβ1-38, Aβ1-40, and Aβ1-42 in a dose-dependent fashion, while levels of sAPPα were conversely increased.
    - In a separate trial called EARLY that was launched in 2015, participants are asymptomatic but at risk of developing Alzheimer’s dementia and were intended to receive drug or placebo once daily for up to 4.5 years with continuous monitoring of cognitive scales.
        - Unfortunately, observation of elevated liver enzymes in two patients led Janssen to announce the discontinuation of this trial on 17 May 2018.

    - The main reason for this was the unfavorable benefit–risk ratio associated with the potential risk to patients of severe liver injury. Severe liver toxicity has not yet been seen in BACE1-null mice, but several BACE1 inhibitors, such as LY2886721, have been found to cause abnormal liver function

4. Elenbecestat (E2609):
    - Elenbecestat (E2609), originally developed by Eisai as a small-molecule inhibitor of BACE1, is currently in clinical trials co-developed with Biogen.
    - During preclinical testing in rodents, guinea pigs, and non-human primates, elenbecestat was shown to strongly reduce CSF and plasma Aβ levels
    - A phase I trial reported that a single dose of 50 mg in 73 healthy participants (either gender, from age 30 to 85 years in six separate cohorts) was well-tolerated and safe.
    - “A single oral ascending-dose study of 5–800 mg and a 14-day multiple oral ascending-dose study of 25–400 mg showed that elenbecestat could significantly reduce plasma or CSF Aβ levels by as much as 92%: plasma Aβ relative to baseline was 52% at 5 mg and 92% at 800 mg”
    - Participants reported adverse effects such as headache and dizziness.
    - No alarming safety concerns were reported apart from relapse of orolabial herpes in the 200 mg cohort.
    - In addition, by comparing different doses to placebo in mild cognitive impairment/prodromal patients or two doses in subjects with mild AD dementia, elenbecestat was found to delay clinical symptoms at the endpoint of the trial
    - A phase IIa trial concluded that of elenbecestat 50 mg/day was safe and consistently reduced CSF Aβ by about 70%.
    - Thus, a standard phase III trial called MISSION AD was initiated in November 2016 and MISSION AD2 was initiated in January 2017, enrolling a total of 1330 early AD subjects.
    - Encouragingly, and unlike earlier mentioned discontinued trials, Biogen announced in June 2018 that the 18-month-long phase II study had revealed less decline in functional cognition in addition to a significant reduction in Aβ levels, quantified by amyloid PET imaging, in patients with mild to moderate form of AD
    - However, they did disclose adverse effects such as upper respiratory tract infection, abnormal dreams and nightmares, contact dermatitis, headache, diarrhea, and falls.
    - This news provides great relief as different BACE1 inhibitors are showing promise.

- According to Eisai, no depigmentation due to BACE 2 inhibition was seen in either animal studies or clinical trials so far.

Observation from study so far/ Why aren't these drugs working:

- BACE 2 is directly related to hair pigmentation
- Most BACE 1 drugs are safe in terms of Phase 1 trials
- Drugs have improved AB 40/42 levels in CSF, and plasma.
- BACE inhibitor was effective in mice/ animal brains
- Most drugs were discontinued in phase 2/3
- Even the slightest level of cognitive symptoms of AD might be too late for BACE inhibitors to work, looking at how most of the trials were discontinued due to inefficiency of drugs.
- **BACE inhibitors need to be administered long before mild symptoms of AD are observed. They need to be given several years before onset symptoms occur.**

BACE inhibitors may not be the most effective way of treatment if they alone are unable to reverse/ treat the cognitive symptoms as well as other deficits in the brain.

A combination of drugs including cholinesterase inhibitors/ Memantine as well as other drugs might be a better/ more effective combination provided these drugs are compatible with one another and are administered slowly.

Updated Question: Will a combination therapy be more effective compared to single drug therapy in Alzheimer's Disease?

Updated Hypothesis: A combination of approaches that target more than one cause of AD such as neuro-inflammation, misfolded proteins, amyloid-beta plaques, and mitochondrial dysfunction will be most effective in the treatment of Alzheimer's Disease.

What is Neuro-Inflammation?

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5909703/#:~:text=In%20Alzheimer's%20disease%20(AD)%20neuroinflammation,plaques%20and%20tangles%20themselves%201.

In Alzheimer's disease (AD) neuroinflammation, instead of being a mere bystander activated by emerging senile plaques and neurofibrillar tangles, contributes as much or more to the pathogenesis as do the plaques and tangles themselves.

- Microglia are phagocytes in the Central nervous system (CNS).
    - They constantly survey their assigned brain regions using their highly motile processes for the presence of pathogens and cellular debris, and simultaneously providing factors that support tissue maintenance
    - They contribute to the protection and remodeling of synapses for proper maintenance and plasticity of neuronal circuits.
    - Once activated by pathological triggers, like neuronal death or protein aggregates, microglia extend their processes to the site of injury, later start migrating to the lesion(wound), and initiate an immune response.
    - In AD, microglia are able to bind to soluble amyloid β (Aβ) oligomers and Aβ fibrils via receptors.
    - The binding of Aβ with CD36, TLR4 and TLR6 results in activation of microglia which start to produce proinflammatory cytokines and chemokines.
        - Cytokines: cell signalling molecules that aid cell to cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma.
        - Chemokines: Cytokine is a general term used for all signalling molecules while chemokines are specific cytokines that function by attracting cells to sites of infection/inflammation.
- Microglia start to engulf Aβ fibrils by phagocytosis.
- In sporadic cases of AD, inefficient clearance of Aβ has been identified as a major pathogenic pathway.
- It has been suggested, that increased cytokine levels are responsible for the insufficient microglial phagocytic capacity by downregulating Aβ phagocytosis receptors

Neuroinflammation- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4321665/

- Neuroinflammation is defined as the brain's activation of the innate immune system, and its main function is to protect the central nervous system (CNS) against infectious insults, injury, or disease.
- It is a complex response involving a host of cellular and molecular changes, recruitment of peripheral immune cells, induction of some intracellular signaling pathways, and release of inflammatory mediators in the brain.
    - All these factors can contribute to the occurrence of neuronal dysfunction and death in AD, either alone or in combination
- Astrocytes and microglia are the major types of glial cells in the CNS, and their activation involves various types of neurodegenerative processes.
    - Reactive glial cells are closely associated with plaques and parallel tangles in AD.

    - Once activated, their processes become hypertrophied; both astrocytes and microglia produce multiple inflammatory factors, including cytokines, prostanoids, chemokines, reactive oxygen species, and cyclooxygenase-(COX)2.
    - Similarly, as indications of neuroinflammation, elevated levels of inflammatory cytokines are discovered in AD.
- As the primary regulators of inflammation in the CNS, the activation of microglia can quickly lead to the release of inflammatory cytokines. In culture, it has been shown that microglia can release several potentially cytotoxic substances, such as cytokines, nitric oxide, arachidonic acid derivatives, proteases, reactive oxygen intermediates, excitatory amino acids, and various neurotrophic factors.
- Neuroinflammation is a process regulated by brain resident macrophages, the microglia cells, which are required to recognize and eliminate any toxic component in the central nervous system (CNS)
- Microglia has a high capacity for mobility, and they can switch between two different phenotypes, M1 and M2, characterized by a different morphology and cytokine profile.
- The M2 phenotype is the resting type that actively monitors the brain in healthy conditions. The switch to M1 begins with the recognition of the pathogen-associated molecular patterns (PAMPs) or the damage-associated molecular patterns (DAMPS) by the pattern recognition receptors (PRRs).
- The activation of this system, the so-called inflammasome, initiates the inflammatory cascade, which results in the secretion of several pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukins 1β, 6 and 18 (IL-1β, IL-6 and IL-18, respectively)
- Pro-inflammatory cytokines' purpose is to orchestrate the neutralization and elimination of toxic molecules and/or cellular debris.
- In normal conditions, once the toxic stimuli have been cleared, microglia swifts to the anti-inflammatory (M1) phenotype and secretes anti-inflammatory cytokines
- Therefore, the chronic neuroimmune system activation underlies the initiation and progression in many dementias, and surely, is involved in the late onset of AD
- Not only Aβ activates the microglia [24], but also misfolded Tau interaction with microglia triggers inflammation
- The elimination of the microglial receptor, NLR family pyrin domain containing 3 (NLRP3) has shown to reduce brain Aβ levels in rodent models of AD
- In addition to the neurological symptoms, neuroinflammation also underlies the psychiatric signs associated with AD, and for that reason, targeting neuroinflammation has also been proposed to treat those comorbid disturbances

- According to the neuroinflammation hypothesis underlying AD, there is a lower incidence of AD among users of chronic non-steroidal anti-inflammatory molecules (NSAIDs)
- NSAIDs inhibit mostly the cyclooxygenase (COX) activity, which synthesizes prostaglandin (PG) from arachidonic acid.
- Anti-inflammatory compounds, inhibiting COX activity, Naproxen and Celecoxib have been tested in clinical trials against AD.
- Naproxen, a non-selective COX inhibitor was administered (220 mg/twice day for two years) to 195 pre-symptomatic AD subjects (aged 55+) with a familial history of AD. The progression of the disease was evaluated with the Alzheimer's Progression Score (APS). Naproxen reduced the rate of the APS, though not significantly
- Celecoxib, a selective COX-2 inhibitor, was administered (200 mg/twice day for 2 years) in 677 pre-symptomatic subjects (70+) with at least one first-degree relative with AD. No improvement in the cognitive symptoms in the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT) in the AD patients compared to the placebo group was found.
- None of these clinical trials analyzed inflammation biomarkers; therefore, these studies cannot test the neuro-inflammation hypothesis underlying AD progression. In addition, these clinical data would shift the focus to different inflammation pathways, other than the COX-PG pathway
- Furthermore, the specific TNF-α inhibitor, Etanercept, was evaluated in a small group of 41 AD patients (55+) with mild to severe AD (SMMSE score between 10 and 27), to test its anti-inflammatory effect and subsequent improvement of cognitive function.
    - The weekly 50 mg subcutaneous administration was well tolerated; however, after 24 weeks of treatment, Etanercept did not show significant beneficial effects in cognition, behavior, systemic cytokine levels or global function compared to the placebo-treated group
- The failure of this clinical trial involves many factors, including insulin resistance thus inhibiting specifically the TNF-α action may not be sufficient to counteract the inflammasome activity, and hence, to effectively prevent disease, perhaps due to the short period of time of assays.

However, under pathological conditions, microglia cells do not go back to their resting state, thus causing a chronic inflammation process, with the overproduction of pro-inflammatory cytokines and reduction of neuroprotective factors that in sustained situations become highly toxic, leading to neurodegeneration

https://www.youtube.com/watch?v=NCfYGzo7HXU

Neuroinflammation is defined as an inflammatory response within the brain or spinal cord. This inflammation is mediated by the production of cytokines, chemokines, reactive oxygen species, and secondary messengers.

Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage.

- Neuroinflammation control with varying combinations of low-dose corticosteroids, anti-inflammatories, microglial suppressors, and nutritional supplements. Spinal fluid flow exercises including walking arm swings, upper body gyration, and deep breathing.
    - Corticosteroids: Corticosteroids are a class of drug that lowers inflammation in the body. They also reduce immune system activity. They ease swelling, itching, redness, and allergic reactions. Doctors often prescribe them to help treat diseases like: asthma.
- Mitochondrial Dysfunction:
    - Mitochondrial dysfunction occurs when the mitochondria don't work as well as they should due to another disease or condition.
    - Many conditions can lead to secondary mitochondrial dysfunction and affect other diseases, including Alzheimer's disease, muscular dystrophy, Lou Gehrig's disease, diabetes and cancer.
    - In most people, primary mitochondrial disease is a genetic condition that can be inherited (passed from parents to their children) in several ways.
    - Loss of function in mitochondria, the key organelle responsible for cellular energy production, can result in the excess fatigue and other symptoms that are common complaints in almost every chronic disease.
    - At the molecular level, a reduction in mitochondrial function occurs as a result of the following changes:
        - 1. a loss of maintenance of the electrical and chemical transmembrane potential of the inner mitochondrial membrane,
        - 2.alterations in the function of the electron transport chain, or
        - 3. a reduction in the transport of critical metabolites into mitochondria.
    - These changes result in a reduced efficiency of oxidative phosphorylation and a reduction in production of adenosine-5′-triphosphate (ATP).

- Mitochondrial dysfunction, characterized by a loss of efficiency in the electron transport chain and reductions in the synthesis of high-energy molecules, such as adenosine-5′-triphosphate (ATP), is a characteristic of aging, and essentially, of all chronic diseases.
- These diseases include neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and many more
- It is well known among researchers that mitochondrial genetic or primary mitochondrial disorders contribute to mitochondrial dysfunction and secondary or acquired degenerative disorders
- Mitochondrial dysfunction arises from an inadequate number of mitochondria, an inability to provide necessary substrates to mitochondria, or a dysfunction in their electron transport and ATP-synthesis machinery.
- The ability of cells to produce almost all high-energy molecules such as ATP is directly related to the ability of mitochondria to 1 convert the energy of metabolites to reduced nicotinamide adenine dinucleotide (NADH) and 2 transfer electrons from NADH to the electron transport chain and eventually to molecular oxygen while pumping protons from the mitochondrial matrix across the inner mitochondrial membrane to the intermembrane space
- This process creates a transmembrane proton gradient and an electrochemical gradient across the mitochondrial inner membrane
- The transmembrane potential created by the proton gradient then uses ATP synthase to flow protons back across the inner mitochondrial membrane and employs the energy from this process to drive adenosine diphosphate (ADP) phosphorylation to ATP
- the consequence of the electron transport process is the production of reactive oxygen species (ROS), highly reactive free radicals that are produced as a by-product of oxidative phosphorylation.
- The main sources of ROS and the related reactive nitrogen species (RNS) are mitochondria, and these free radicals can damage cellular lipids, proteins, and DNA.
- However, some mechanisms can neutralize ROS/RNS; dismutase enzymes and antioxidants can control excess amounts of ROS/RNS.
- In addition to creation of ROS/RNS, the electron transport process can induce uncoupling proteins, resulting in a controlled leak of protons back across the proton gradient of the inner mitochondrial membrane into the mitochondrial matrix
- This leak results in reduced ATP production while it still consumes excess oxygen

    - Mitochondrial dysfunction is directly related to excess fatigue
    - Fatigue is considered a multidimensional sensation that is perceived to be a loss of overall energy and an inability to perform even simple tasks without exertion
    - Although mild fatigue can be caused by a number of conditions, including depression and other psychological conditions, moderate to severe fatigue involves cellular energy systems
    - At the cellular level, moderate to severe fatigue is related to loss of mitochondrial function and diminished production of ATP
    - Intractable fatigue lasting more than 6 months that is not reversed by sleep (chronic fatigue) is the most common complaint of patients seeking general medical care.
    - As a result of aging and chronic diseases, oxidative damage to mitochondrial membranes impairs mitochondrial function
    - As an example, individuals with chronic fatigue syndrome present evidence of oxidative damage to DNA and lipids such as oxidized blood markers and oxidized membrane lipids that is indicative of excess oxidative stress.
    - A number of natural supplements have been used to treat non psychological fatigue and mitochondrial dysfunction.
    - These supplements include those containing vitamins, minerals, antioxidants, metabolites, enzyme inhibitors and cofactors, mitochondrial transporters, herbs, and membrane phospholipids

An alternative to the amyloid cascade hypothesis, the mitochondrial cascade hypothesis, sees brain aging and AD as part of a continuum. This continuum is influenced by common underlying mechanisms. The mitochondrial cascade hypothesis proposes mitochondrial function declines with age, and this decline promotes some features of brain aging including Aβ accumulation. When mitochondrial decline surpasses a threshold compensatory mechanisms fail and late-onset AD ensues. In contrast to the amyloid cascade hypothesis, which views Aβ as the primary upstream AD biomarker, the mitochondrial cascade hypothesis in general sees Aβ more as a downstream biomarker of brain aging. If the mitochondrial cascade hypothesis is correct, treatments designed to improve mitochondrial function should benefit late-onset AD patients more than treatments designed to directly reduce Aβ.

- Misfolded Proteins:
    - A protein starts off in the cell as a long chain of, on average, 300 building blocks called amino acids.
    - There are 22 different types of amino acids, and their ordering determines how the protein chain will fold upon itself.

- Some regions of the protein chain coil up into slinky-like formations called “alpha helices,” while other regions fold into zigzag patterns called “beta sheets,” which resemble the folds of a paper fan.
- These two structures can interact to form more complex structures.
- These complex structures allow proteins to perform their diverse jobs in the cell.
- By folding into distinct shapes, proteins can perform very different roles despite being composed of the same basic building blocks.
- Folding allows a protein to adopt a functional shape, but it is a complex process that sometimes fails. Protein folding can go wrong for three major reasons:
    - 1: A person might possess a mutation that changes an amino acid in the protein chain, making it difficult for a particular protein to find its preferred fold or “native” state. These mutations are located in the DNA sequence or “gene” that encodes one particular protein. Therefore, these types of inherited mutations affect only that particular protein and its related function.
    - 2: On the other hand, protein folding failure can be viewed as an ongoing and more general process that affects many proteins. When proteins are created, the machine that reads the directions from DNA to create the long chains of amino acids can make mistakes. Scientists estimate that this machine, the ribosome, makes mistakes in as many as 1 in every 7 proteins! These mistakes can make the resulting proteins less likely to fold properly.
    - 3: Even if an amino acid chain has no mutations or mistakes, it may still not reach its preferred folded shape simply because proteins do not fold correctly 100% of the time. Protein folding becomes even more difficult if the conditions in the cell, like acidity and temperature, change from those to which the organism is accustomed.
- There are two completely different problems that occur in cells when their proteins do not fold properly.
- One type of problem, called “loss of function,” results when not enough of a particular protein folds properly, causing a shortage of “specialized workers” needed to do a specific job
    - For example, imagine that a properly folded protein is perfectly shaped to bind a toxin and break it into less toxic byproducts. Without enough of the properly folded protein available, the toxin will build up to damaging levels.

- Proteins that fold improperly may also impact the health of the cell regardless of the function of the protein.
    - When proteins fail to fold into their functional state, the resulting misfolded proteins can be contorted into shapes that are unfavorable to the crowded cellular environment.
    - Most proteins possess sticky, “water-hating” amino acids that they bury deep inside their core. Misfolded proteins wear these inner parts on the outside, like a chocolate-covered candy that has been crushed to reveal a gooey caramel center. These misfolded proteins often stick together forming clumps called “aggregates.” Scientists hypothesize that the accumulation of misfolded proteins plays a role in several neurological diseases, including Alzheimer’s, Parkinson’s, Huntington’s, and Lou Gehrig’s (ALS) disease, but scientists are still working to discover exactly how these misfolded, sticky molecules inflict their damage on cells.
- How do our cells protect themselves from misfolded proteins?
    - cells are accustomed to coping with this problem and have several systems in place to refold or destroy aberrant protein formations.
    - Chaperones are one such system. Appropriately named, they accompany proteins through the folding process, improving a protein’s chances of folding properly and even allowing some misfolded proteins the opportunity to refold
    - Some cater specifically to helping one type of protein fold, while others act more generally. Some chaperones are shaped like large hollow chambers and provide proteins with a safe space, isolated from other molecules, in which to fold. Production of several chaperones is boosted when a cell encounters high temperatures or other conditions making protein folding more difficult, thus earning these chaperones the alias, “heat shock proteins.

Combination Therapy :

- Treating dementia has become a major challenge in clinical practice. Presently, acetylcholinesterase inhibitors are the first-line drugs in the treatment of Alzheimer’s disease (AD). These options are now complemented by memantine, which is approved for the treatment of moderate-to-severe AD.
- Altogether, a minimum of six agent classes already exist,
- These include cholinesterase inhibitors, blockers of the NMDA receptor, antioxidants or blockers of oxidative deamination (including Ginkgo biloba),

anti-inflammatory agents, neurotrophic factors (including hormone replacement therapy and drugs acting on insulin signal transduction) and antiamy-loid agents (including cholesterol-lowering therapy).

- Presently, only nine clinical studies have been published that have investigated the effects of a combination regimen on cognitive performance or AD.
- Some studies, however, showed some evidence for synergistic combination effects of symptomatic therapy, including delay or prevention of disease progression in AD patients.
- According to the amyloid-hypothesis several therapeutic strategies have been proposed as potentially primary preventive therapy.
- The first strategy aims at partially inhibiting either of the two proteases, β- and γ-secretase, that generate Aβ from the amyloid precursor protein.
- one attempts to prevent the oligomerization of Aβ or to enhance its clearance from the cerebral cortex. This approach is exemplified by the use of active or passive Aβ immunisation, in which antibodies to Aβ decrease cerebral levels of the peptide.
- Considering the complexity of AD, and that multiple etiologies may contribute to the disease, a combination of therapeutic agents may result in more effective strategies for treatment than one drug, several therapeutic strategies have been alone.
- Cholinesterase Inhibitors:
    - The main drugs that are available for the symptomatic treatment of AD are cholinesterase inhibitors,which increase the availability of acetylcholine at cholinergic synapses.
    - Compared with placebo, positive effects of AChE inhibitors are shown with regard to cognition and global impression of the physician.
    - In placebo-controlled studies, Significant differences in the efficacy of differences in activities of daily living and behaviour shown, mostly in favour of AChE inhibitors.
    - Significant differences in the efficacy of different AChE inhibitors are not known.
    - In patients who experience insufficient effectiveness or intolerable adverse effects, a change from one AChE inhibitor to another may help up to 50% of these patients; the change from one AChE inhibitor to another shows loss of effectiveness over the period between washout and new administration.
- One drug blocking a single step in a complex pathogenic network often cannot block all crucial disease-propagation mechanisms.

- Combination therapy permits deployment of agents that block multiple targets, thus increasing the likelihood of arresting or delaying the pathogenesis of a disease.
- However, as a general summary, a drug that fails as monotherapy may be of benefit as a combination regimen.
- Also, in contrast to additive and synergistic beneficial effects, drugs in combination may show additive or synergistic adverse effects and/or unexpected antagonistic effects.

Considering that single drug therapies (monotherapies) have not yet proven to be effective in the treatment of Alzheimer's, a combination of drugs/ drug types might be a better solution. There would need to be many clinical trials needed to be done to prove the efficacy of the drugs and their compatibility together.

List of Possible Drugs/Drug types that would be used in a combination:

Cholinesterase inhibitors: can slightly delay the loss of brain function in people who have mild to moderate Alzheimer's disease. In people with advanced Alzheimer's disease, certain nerve cells are much less active. This means that it takes longer for brain signals to be sent. Cholinesterase inhibitors aim to increase communication between the nerve cells to try to improve the symptoms of Alzheimer's. These drugs have been approved for use in mild to moderate Alzheimer's disease. In Germany, three different cholinesterase inhibitors are currently available: donepezil, galantamine and rivastigmine. They are taken in the form of tablets. Rivastigmine is also available in a patch. Here the drug is absorbed into the body through the skin.

The studies show that the cholinesterase inhibitors donepezil, galantamine and rivastigmine can slightly delay the loss of mental abilities in people who have mild to moderate Alzheimer's disease. For instance, some of the people with Alzheimer's who regularly took one of these medications were able to remember things more easily.All three medications can cause side effects like nausea, vomiting, loss of appetite, dizziness or diarrhea. Some people might stop taking them as a result. Higher doses are more likely to cause side effects. Depending on which medication they take, about 1 to 3 out of 10 people feel nauseous or vomit.

Cholinesterase inhibitors work by increasing levels of acetylcholine, a chemical messenger involved in memory, judgment and other thought processes. Certain brain cells release acetylcholine, which helps deliver messages to other cells. After a message reaches the receiving cell, various other chemicals, including an enzyme called acetylcholinesterase, break acetylcholine down so it can be recycled. Alzheimer's disease damages or destroys cells that produce and use acetylcholine, thereby reducing the amount available to carry messages. A cholinesterase inhibitor slows the breakdown of acetylcholine by blocking the activity of acetylcholinesterase. By maintaining acetylcholine levels, the drug may help compensate for the loss of functioning brain cells.

Galantamine appears to stimulate the release of acetylcholine and strengthen the way certain message-receiving nerve cells respond to it. Rivastigmine may block the activity of another enzyme involved in breaking down acetylcholine.

In clinical trials of all three cholinesterase inhibitors, people taking the medications performed better on memory and thinking tests than those taking a placebo, or inactive substance. However, the degree of improvement was small. In terms of overall effect, cholinesterase inhibitors may delay or slow worsening of symptoms. The effectiveness of cholinesterase inhibitors, as well as how long they are effective, varies from person to person.

If side effects occur, they commonly include nausea, vomiting, loss of appetite and increased frequency of bowel movements. It is strongly recommended that a physician who is experienced in using these medications monitor patients who are taking them and that the recommended guidelines be strictly observed.

Memantine: Memantine (Namenda®) is prescribed to improve memory, attention, reason, language and the ability to perform simple tasks. It was the first Alzheimer's drug of the NMDA receptor antagonist type approved in the United States. It is used to treat moderate-to-severe Alzheimer's. The FDA declined to approve memantine for mild Alzheimer's in 2005.

Memantine appears to work by regulating the activity of glutamate, a chemical involved in information processing, storage and retrieval. Glutamate plays an essential role in learning and memory by triggering NMDA receptors to let a controlled amount of calcium into a nerve cell. The calcium helps create the chemical environment required for information storage. Excess glutamate, on the other hand, overstimulates NMDA receptors so that they allow too much calcium into the nerve cells. That leads to disruption and death of cells. Memantine may protect cells against excess glutamate by partially blocking NMDA receptors.

One clinical study showed that people taking memantine showed a small but statistically significant improvement in their mental function and ability to perform daily activities. But study participants with the lowest cognitive functioning showed no improvement on either daily activities or overall function. Another study randomly assigned participants to receive either 10 mg of memantine twice a day or a placebo in addition to donepezil (Aricept), a cholinesterase inhibitor. Those receiving memantine showed a statistically significant benefit in mental functioning and performing daily activities, while participants taking donepezil plus placebo continued to decline.Adverse side effects include headache, constipation, confusion and dizziness.

Combination of memantine and donepezil: Namzaric® , a combination of donepezil and memantine, was approved by the FDA for the treatment of moderate-to-severe Alzheimer's in people who are taking donepezil hydrochloride 10 mg.

Namzaric may cause serious side effects, including: Muscle problems in patients given anesthesia. Slow heartbeat and fainting: This happens more often in people with heart problems.Increased stomach acid: This raises the chance of ulcers and bleeding, especially when taking Namzaric. Nausea and vomiting. Difficulty passing urine. Seizures. Worsening of lung problems in people with asthma or other lung disease

Individuals taking Namzaric may see an improvement in cognition and overall mental function, and a temporary slowdown in the worsening of symptoms. However, there is no evidence that Namzaric prevents or slows the underlying disease process in patients with Alzheimer's disease.

Non-Steroidal Anti-Inflammatory Agents (NSAID): Inflammatory processes Involving cytokines and Glia cells play an important and complex role in the pathogenesis of Alzheimer's. In the Border zone of early diffuse plaques astrocytes can be found functioning as antigen-presenting cells under specific conditions. In the stage no signs of neurodegeneration are parent but primary synaptic changes are perceptible. As a result the membrane attack complex is activated damaging the Integrity of the cell membrane. In addition to the anti-inflammatory effect NSAIDs direct effect on amyloid formation could be relevant for selecting them as potential treatment in AD. The recognition that NSAIDs can bind to and activate the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-y Has offered an additional explanation for the action of these drugs in Alzheimer's. Ppar-y agonists were shown to play a critical role in regulating the inflammatory responses of microglia and monocytes to AB.To date, treatment of patients in the stage of manifest Alzheimer's disease has been without success. Gastrointestinal adverse effects limit those results so that in general, NSAIDs cannot be recommended for treatment of mild to moderate Alzheimer's.

Cholesterol lowering therapy: the production amyloid-beta depends on the availability of cholesterol in the nerve cells. Balance of the alpha and beta secretase activity is linked with cellular lipid composition. High cellular cholesterol levels increase amyloidogenic processing of amyloid precursor protein by beta-secretase whereas low levels of cholesterol levels increase the physiological metabolism of APP by a-secretase. By this mechanism, a depletion of cholesterol from neuronal membranes could be a therapeutic approach for the treatment of AD. Epidemiological studies have shown that treatment with cholesterol-lowering drugs reduce the prevalence of Dementia in comparison with normal population.

- Treatment combinations can be characterized as pharmacodynamic or pharmacokinetic.
- Pharmacodynamic combinations are designed to exert multiple effects on disease biology; pharmacokinetic combinations affect a drug's absorption, distribution, metabolism, or elimination
- Pharmacodynamic combinations for treatment of AD can include symptomatic agents that address the behavioral and cognitive symptoms of AD without changing the underlying disease biology or disease-modifying therapies (DMTs) that change disease course by addressing the underlying biology that leads to nerve cell death
- Combination therapy can be used flexibly in addressing the drug target, delivery method, or delivery timing. For example, within AD DMT development programs, therapeutic agents could target amyloid, tau, or other disease processes, such as inflammation.
- Sequential combinations address targets consecutively (e.g., remove amyloid plaque with monoclonal antibody and then follow with a BACE 1 inhibitor to prevent plaque reaccumulation) . Sequential combinations will likely evolve in concert with increased understanding of AD etiology and reflect the complexity of the biology of AD
- Verubecestat had demonstrated promising findings in a phase I trial by reducing Aβ40 and Aβ42 in the cerebrospinal fluid of healthy subjects and patients with mild to moderate AD. Verubecestat was also investigated in patients with mild to moderate AD, but the development program was terminated because of a lack of positive effect in an interim analysis of the trial (NCT01739348)
- This lack of efficacy supports the theory that use of a BACE 1 inhibitor in patients who have accumulated enough Aβ deposition to have dementia is unlikely to have clinical benefit.
- Another method for targeting the amyloid cascade is the use of humanized or fully human monoclonal antibodies (mAbs) that bind and mount an immunologic response against the Aβ peptide, leading to increased amyloid clearance. Based

on promising results in phase I/II trials, three Aβ mAbs (aducanumab, gantenerumab, and crenezumab) are being investigated in placebo-controlled phase III trials as add-on therapy in patients with early (i.e., prodromal) or mild AD. These trials are estimated to be completed between 2019 and 2022.

The second major pathologic hallmark of AD is the formation of intracellular neurofibrillary tangles composed of hyperphosphorylated tau. Tau pathology is characterized primarily by abnormal phosphorylation and other modifications that alter tau structure and lead to formation of tau protein aggregates, associated with neurofibrillary degeneration and dementia [7]. Tau aggregation inhibitors (TAIs) have the potential to prevent or reverse tau aggregation and consequently reduce tau pathology and associated behavioral deficits in patients with AD.

“There are two DMTs currently in phase III trials that address two targets and represent valid combination therapies: ALZT-OPT1 and Gamunex (immune globulin intravenous (human), 10%; Grifols Therapeutics, Clayton, NC, USA). The ALZT-OPT1 trial, a combination regimen with cromolyn (anti-amyloid agent) and ibuprofen (anti-inflammatory agent), is enrolling patients with early AD who are either receiving or not receiving standard-of-care agents. Cromolyn is a treatment for asthma approved by the US Food and Drug Administration (FDA) that bears structural similarity to other anti-amyloid agents and is likely to cross the blood-brain barrier. Cromolyn reduced Aβ fibrilization and oligomerization *in vitro* and reduced $A\beta_{40}$ and $A\beta_{42}$ monomer concentrations in the mouse brain; oligomerization and fibrillation were unchanged *in vivo*. ALZT-OPT1 is a true combination trial in that the combination targets multiple disease pathways (amyloid and inflammation) and includes multiple methods of administration (intranasal inhaler for cromolyn and oral tablet for ibuprofen). ALZT-OPT1 is also an add-on study because it allows patients to continue standard-of-care treatments on stable doses.”

Although single agent therapy has the advantage of simplicity, fostering patient compliance and allowing straightforward identification of the source of adverse effect, monotherapy also has substantial limitations. Many diseases are a product of multiple pathophysiological pathways. One drug blocking a single step in a complex pathogenic network often cannot block all crucial disease-propagating mechanisms.

Inhibition of amyloid formation and clearance of existing amyloid have also been achieved with anti-Aβ antibodies. Phase 3 clinical trials with bapineuzumab and solanezumab have been completed recently. Although the studies failed to demonstrate an effect on the primary endpoints, some encouraging signs on cognitive, functional, and biomarker measures have been noted.

Given the complexity of AD, treatment of patients remains challenging. The currently approved treatments for AD are limited to cholinesterase inhibitors and memantine or the combination of these agents.

The high failure rate of the therapies in development for AD stems in large part from the complex pathologic causes of the disease, as well as our incomplete understanding of the relationships among the numerous pathways involved in development of AD and subsequent neurodegeneration, and the potential lack of efficacy of available agents.

Combining therapeutic agents may allow for lower doses of the individual agents, reducing costs and side effects. Innovative and adaptive clinical trial designs may also capture the potential evolution of therapeutic combinations over the long and complex course of disease progression, with one set of agents appropriate for preclinical AD, another for early-stage AD, and yet another for AD dementia.

Antibodies which target existing Aβ species act downstream of BACE1 inhibitors. We therefore evaluated whether combined pharmacological intervention with a BACE1 inhibitor and a plaque specific antibody would lead to an enhanced amyloid-lowering effect.

Problem

Alzheimer's disease is known to affect one in 10 people ages 65 and older. As per WHO, 50 million people aged 65 and older are living with Alzheimer's or other pathologies involving dementia worldwide. This number is expected to double by 2030. These statistics are estimated to rapidly increase as the human lifespan increases. Currently it can be neither prevented nor reversed or treated. The problem is that there are no effective treatments for the disease. There are few FDA approved medications and drugs that treat the symptoms of Alzheimer's however there are no drugs that can reverse, prevent, or treat it.

Upon studying the pathophysiology of this disease, I came to know that the formation of Amyloid-beta plaques that are caused by the cleavage of the Amyloid Precursor Protein (APP) by the beta-secretase is the root cause of the development and progression of Alzheimer's. None of the existing approved treatment options are targeting this cause. Clinical trials for BACE inhibitors have so far not yielded positive results that can allow their use for treatment on the general population. My project focuses on a different therapeutic approach that involves a combination of drugs that target more than one aspect of the disease such as neuro-inflammation, misfolded proteins, amyloid-beta plaques, and mitochondrial dysfunction for the treatment of Alzheimer's Disease.

Research

What is AD?

The brain relies on three key processes to survive.

The first is communication. Your brain is sending signals to other neurons and receiving signals from others. This is done through chemical messengers in your brain called neurotransmitters. These signals contain instructions on what to do and is what tells the body to respond to stimuli. When a neuron receives signals from other neurons, it generates an electrical charge that travels down the length of its axon and releases neurotransmitter chemicals across a tiny gap, called a synapse. Neurotransmitters are chemical messengers that transmit a message from a nerve cell across the synapse to a target cell. The target can be another nerve cell, or a muscle cell, or a gland cell. They are chemicals made by the nerve cell specifically to transmit the message. Neurotransmitters are released from synaptic vesicles in synapses into the synaptic cleft, where they are received by neurotransmitter receptors on the target cell. Each neurotransmitter molecule then binds to specific receptor sites on a dendrite of a nearby neuron. This process triggers chemical or electrical signals that either stimulate or inhibit activity in the neuron receiving the signal.

The second is Metabolism. It is the breaking down of chemicals and nutrients within a cell. To perform this function, cells require energy in the form of oxygen and glucose, which are supplied by blood circulating through the brain. The brain actually needs the most energy out of all other organs and the richest blood supply in the entire body because they need to send signals. The third key process is repair, remodel, and regenerate. Neurons live for 100 years in our brains so we need to constantly maintain and repair them. Neurons also continuously adjust, or “remodel,” their synaptic connections depending on how much stimulation they receive from other neurons. Remodeling of synaptic connections and neurogenesis are important for learning, memory, and possibly brain repair.

A healthy brain shrinks in aging but doesn't lose neurons. In Alzheimer’s disease damage is widespread as many neurons stop functioning, lose connections with other neurons, and die. At first, Alzheimer’s disease typically destroys neurons and their connections in parts of the brain involved in memory, including the entorhinal cortex and hippocampus. Then affects areas in the cerebral cortex responsible for language, reasoning, and social behavior. Ultimately, the disease is fatal.

Alzheimer’s disease is named after Dr. Alois Alzheimer who in 1906, noticed changes in the brain tissue of a woman who had died of an unusual mental illness. She had symptoms like memory loss, language problems, and unpredictable behavior. After she died, he examined her brain and found many clumps now referred to as amyloid-beta plaques which are insoluble proteins that accumulate because waste microglia cells and astrocytes cannot process and remove it. The second cause was tangles of fibers now called tau, or neurofibrillary tangles. These plaques build up between neurons, blocking pathways through which the communication happens. Without direction, neurons are

helpless, they don't receive energy, and they can't repair themselves so they eventually die. Your brain loses connection with the rest of your body causing memory loss, speech impairment, and eventually the ability to be physically active.

How are amyloid-beta plaques and neurofibrillary tangles created?

Amyloid-beta comes from this larger protein called Amyloid Precursor Protein (APP). APP plays roles both as an intact membrane protein and when broken into pieces. The intact protein is a receptor protein that sends signals through the G-protein system. APP also binds to many structural molecules outside cells, such as heparin and laminin, so it may play a role in cell adhesion. APP is also broken into several functional fragments by a set of dedicated proteases, termed secretases. A protease is an enzyme which breaks down proteins and peptides. Secretases are enzymes that "snip" pieces off a longer protein that is embedded in the cell membrane. Among other roles in the cell, secretases act on the Amyloid Precursor Protein (APP) to cleave the protein into three fragments.

The brain has APP which is cleaved by 3 secretases: alpha, beta, gamma. In a normal brain, the alpha secretase acts on APP and cleaves of sAPPa (secreted APP alpha). What is left is an 83 amino acid long membrane-bound C-terminal fragment called CTF83. In a brain with Alzheimers's, an enzyme called beta-secretase acts on APP and cleaves it into sAPPb (secreted APP beta). What is left is a 99 amino acid long membrane-bound c-terminal fragment called CTF99. In normal signaling, CTF83 is further cleaved by the gamma-secretase complex made up of PSEN1, Pen-2, APH-1, GSAP, and NCT. Cleavage of CTF83 leads to the generation of APP intracellular domain (AICD fragment). AICD fragment translocates to the nucleus where it affects the transcriptional regulation of several proteins, and drives neuroprotective pathways. It is also important to know that sAPP alpha gets secreted from the neurons and drives normal synaptic signaling leading to synaptic plasticity, learning and memory, neuronal survival, and emotional behaviours. In AD, gamma-secretase complex is also assembled but cleaves CTF99 fragment into an AICD fragment and an abeta 40/42 peptide. AICD is again translocated to the nucleus where it affects transcriptional regulation of several proteins and drives neuroprotective pathways. The Abeta 40/42 peptide however is involved in downstream pathways related to AD. This peptide misfolds resulting in a sticky exterior that attaches to other proteins as well as other abeta peptides forming amyloid-beta plaques.

Tau is a protein that helps stabilize the internal skeleton of nerve cells (neurons) in the brain. This internal skeleton has a tube-like shape through which nutrients and other essential substances travel to reach different parts of the neuron. In Alzheimer's

disease, an abnormal form of tau builds up and causes the internal skeleton to fall apart.

Tau accumulation has been shown to promote brain cell damage and death in Alzheimer’s and other dementias, but the exact processes that lead to this toxicity are unclear. Some studies suggest stress in the brain cell's endoplasmic reticulum (ER), the part of the cell where proteins are produced, may play a role in the toxic effect of tau tangles and abnormal tau. Results of these studies may clarify how tau toxicity develops in the brain and promotes brain cell damage in Alzheimer's. The results may also suggest new avenues to investigate for therapies that may prevent or slow the brain changes of Alzheimer's and other dementias.

What treatment strategies are being implemented?

Treating dementia has become a major challenge in clinical practice. Presently, acetylcholinesterase inhibitors are the first-line drugs in the treatment of Alzheimer’s disease (AD). These options are now complemented by memantine, which is approved for the treatment of moderate-to-severe AD. Cholinesterase inhibitors aim to increase communication between the nerve cells to try to improve the symptoms of Alzheimer’s. Cholinesterase inhibitors work by increasing levels of acetylcholine, a chemical messenger involved in memory, judgment and other thought processes. Certain brain cells release acetylcholine, which helps deliver messages to other cells. After a message reaches the receiving cell, various other chemicals, including an enzyme called acetylcholinesterase, break acetylcholine down so it can be recycled. Alzheimer’s disease damages or destroys cells that produce and use acetylcholine, thereby reducing the amount available to carry messages. A cholinesterase inhibitor slows the breakdown of acetylcholine by blocking the activity of acetylcholinesterase. By maintaining acetylcholine levels, the drug may help compensate for the loss of functioning brain cells. Compared with placebo, positive effects of AChE inhibitors are shown with regard to cognition and global impression of the physician.

These drugs have been approved for use in mild to moderate Alzheimer’s disease. In Germany, three different cholinesterase inhibitors are currently available: donepezil, galantamine and rivastigmine. They are taken in the form of tablets. Rivastigmine is also available in a patch. Here the drug is absorbed into the body through the skin. The studies show that the cholinesterase inhibitors donepezil, galantamine and rivastigmine can slightly delay the loss of mental abilities in people who have mild to moderate Alzheimer’s disease. For instance, some of the people with Alzheimer’s who regularly took one of these medications were able to remember things more easily. In clinical

trials of all three cholinesterase inhibitors, people taking the medications performed better on memory and thinking tests than those taking a placebo, or inactive substance.

The other FDA approved drugs are Memantine. Memantine (Namenda®) is prescribed to improve memory, attention, reason, language and the ability to perform simple tasks. It was the first Alzheimer's drug of the NMDA receptor antagonist type approved in the United States. It is used to treat moderate-to-severe Alzheimer's. Memantine appears to work by regulating the activity of glutamate, a chemical involved in information processing, storage and retrieval. Glutamate plays an essential role in learning and memory by triggering NMDA receptors to let a controlled amount of calcium into a nerve cell. The calcium helps create the chemical environment required for information storage. Excess glutamate, on the other hand, overstimulates NMDA receptors so that they allow too much calcium into the nerve cells. That leads to disruption and death of cells. Memantine may protect cells against excess glutamate by partially blocking NMDA receptors.

One clinical study showed that people taking memantine showed a small but statistically significant improvement in their mental function and ability to perform daily activities. Another study randomly assigned participants to receive either 10 mg of memantine twice a day or a placebo in addition to donepezil (Aricept), a cholinesterase inhibitor. Those receiving memantine showed a statistically significant benefit in mental functioning and performing daily activities, while participants taking donepezil plus placebo continued to decline.

The last FDA approved drug option for Alzheimer's treatment is Namzaric® , a combination of donepezil and memantine, for the treatment of moderate-to-severe Alzheimer's in people who are taking donepezil hydrochloride 10 mg. Individuals taking Namzaric may see an improvement in cognition and overall mental function, and a temporary slowdown in the worsening of symptoms. However, there is no evidence that Namzaric prevents or slows the underlying disease process in patients with Alzheimer's disease.

These drugs can only treat the cognitive symptoms of Alzheimer's. They are unable to prevent, reverse, or treat the disease itself. In order to treat Alzheimer's, the drug needs to target one of the causes of this disease. There are many monotherapeutic approaches that individually target the causes of AD.

Inflammatory processes involving cytokines and Glia cells play an important and complex role in the pathogenesis of Alzheimer's. Chronic inflammation is referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic inflammation vary with the cause of the

injury and the ability of the body to repair and overcome the damage. Neuroinflammation control with varying combinations of low-dose corticosteroids, anti-inflammatories, microglial suppressors, and nutritional supplements. Spinal fluid flow exercises including walking arm swings, upper body gyration, and deep breathing. In the Border zone of early diffuse plaques astrocytes can be found functioning as antigen-presenting cells under specific conditions. In the stage no signs of neurodegeneration are parent but primary synaptic changes are perceptible. As a result the membrane attack complex is activated damaging the Integrity of the cell membrane. In addition to the anti-inflammatory effect NSAIDs direct effect on amyloid formation could be relevant for selecting them as potential treatment in AD. The recognition that NSAIDs can bind to and activate the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-y Has offered an additional explanation for the action of these drugs in Alzheimer's. Ppar-y agonists were shown to play a critical role in regulating the inflammatory responses of microglia and monocytes to AB.

Another monotherapeutic approach targets Cholesterol lowering therapy. The production amyloid-beta depends on the availability of cholesterol in the nerve cells. Balance of the alpha and beta secretase activity is linked with cellular lipid composition. High cellular cholesterol levels increase amyloidogenic processing of amyloid precursor protein by beta-secretase whereas low levels of cholesterol levels increase the physiological metabolism of APP by a-secretase. By this mechanism, a depletion of cholesterol from neuronal membranes could be a therapeutic approach for the treatment of AD.

The next monotherapeutic approach targets the amyloid cascade, BACE inhibitors. BACE inhibitors are drugs to block the beta-secretase enzyme from cleaving the Amyloid-Precursor Protein. It has been shown that deletion of BACE-1 (beta-site amyloid precursor protein cleaving enzyme 1) abrogates AB production and betters cognitive/behavioral deficiencies, as observed in transgenic mice overexpressing human APP with familial AD mutations, indicating that inhibition of BACE1 has a direct effect. A rare human mutation at the BACE1 cleavage site of APP results in a 40% decrease in Aβ production in vitro, a significantly reduced propensity for Aβ to aggregate, a five- to seven-fold reduced risk of developing AD, and greater resilience to cognitive dysfunction in elderly individuals, implying that BACE1 cleavage alone appears to be beneficial in the human brain.

Inhibition of BACE1 directly reduces Aβ-mediated impairments in synaptic transmission. Deletion of BACE1 in mice appears to have a minor impact on mouse growth or overall functions, perhaps related to the fact that most BACE1 substrates are also shed by α-secretase. Direct inhibition of γ-secretase, another strategy to reduce Aβ generation, is now recognized to be more challenging due to the indispensable physiological roles

of γ-secretase, leading investigators and many companies to focus on BACE1 inhibitors, which act upstream of γ-secretase in Aβ generation. Thus, BACE1 is recognized as a better-positioned target for treating AD patients.

Upon studying the clinical trials from BACE inhibitors, my observation was that most BACE 1 drugs are safe in terms of Phase 1 trials. Most BACE inhibitor drugs have improved AB 40/42 levels in CSF, and plasma. They were also effective in mice/ animal brains. Most drugs were discontinued in phase 2 or 3. Even the slightest level of cognitive symptoms of AD might be too late for BACE inhibitors to work, looking at how most of the trials were discontinued due to inefficiency of drugs. **BACE inhibitors need to be administered long before mild symptoms of AD are observed. They need to be given several years before onset symptoms occur.** BACE inhibitors may not be the most effective way of treatment if they alone are unable to reverse/ treat the cognitive symptoms as well as other deficits in the brain.

A combination of drugs including cholinesterase inhibitors/ Memantine as well as other drugs might be a better/ more effective combination provided these drugs are compatible with one another and are administered slowly. The combination of drugs is targeting the decline of cognitive symptoms.

Combination therapy for Alzheimer's.

There are two disease modifying therapies (DMTs) currently in phase III trials that address two targets and represent valid combination therapies: ALZT-OPT1 and Gamunex (immune globulin intravenous (human), 10%; Grifols Therapeutics, Clayton, NC, USA). The ALZT-OPT1 trial, a combination regimen with cromolyn (anti-amyloid agent) and ibuprofen (anti-inflammatory agent), is enrolling patients with early AD who are either receiving or not receiving standard-of-care agents. Cromolyn is a treatment for asthma approved by the US Food and Drug Administration (FDA) that bears structural similarity to other anti-amyloid agents and is likely to cross the blood-brain barrier. Cromolyn reduced Aβ fibrilization and oligomerization *in vitro* and reduced $A\beta_{40}$ and $A\beta_{42}$ monomer concentrations in the mouse brain; oligomerization and fibrillation were unchanged *in vivo*. ALZT-OPT1 is a true combination trial in that the combination targets multiple disease pathways (amyloid and inflammation) and includes multiple methods of administration (intranasal inhaler for cromolyn and oral tablet for ibuprofen). ALZT-OPT1 is also an add-on study because it allows patients to continue standard-of-care treatments on stable doses."

Although single agent therapy has the advantage of simplicity, fostering patient compliance and allowing straightforward identification of the source of adverse effect,

monotherapy also has substantial limitations. Many diseases are a product of multiple pathophysiological pathways. One drug blocking a single step in a complex pathogenic network often cannot block all crucial disease-propagating mechanisms.

Combining therapeutic agents may allow for lower doses of the individual agents, reducing costs and side effects. Innovative and adaptive clinical trial designs may also capture the potential evolution of therapeutic combinations over the long and complex course of disease progression, with one set of agents appropriate for preclinical AD, another for early-stage AD, and yet another for AD dementia.

Data

Data of Individual BACE-1 inhibitors:

https://www.alzforum.org/therapeutics/verubecestat Verubecestat:

- Phase 1 trials tested single doses up to 450 mg and multiple doses from 12 to 150 mg/day.
- Two Phase 1/2 dose-ranging trials further evaluated the tolerability and pharmacology of single and multiple doses, respectively, in 88 healthy adults.
- At the 2012 AAIC conference in Vancouver, Canada, MK-8931 was reported to have been generally safe, without discontinuations due to side effects, and to have reduced CSF Aβ concentration in AD patients.
- These studies used repeated CSF sampling, which found that CSF Aβ was reduced by up to 90 percent (see Jul 2012 conference news)
- In November 2012, Merck started EPOCH, an 18-month Phase 2/3 trial comparing 12, 40, or 60 mg/day of MK-8931 given as once-daily tablets to placebo in people with mild to moderate AD
- EPOCH started out treating 200 people in Phase 2 and, after an interim safety analysis, expanded to Phase 3 with a total of 2,221 participants. This trial included conventional cognitive and functional primary outcomes, as well as substudies for biomarker outcomes indicating changes in brain amyloid, CSF tau levels, and brain volume.
- On 14 February 2017, Merck announced a premature end to this trial following an interim analysis . A subsequent paper further detailed the safety data, noting particularly that while psychiatric side effects did not get worse over time, falls and injuries did
- Although a dramatic reduction of Aβ40, Aβ42, and sAPPβ in CSF of up to 80% was detected and a small reduction in plaque load was confirmed by amyloid PET in participants taking the drug, the clinical trial was terminated in February 2018, with verubecestat exhibiting no improvement in cognitive function in AD patients cited as the reason.

The results of this drug trial suggest that BACE1 inhibitors need to be given several years before the onset of AD symptoms.

Lanabecestat: https://www.alzforum.org/therapeutics/azd3293

- Lanabecestat, a small-molecule, orally administered BACE1 inhibitor developed by AstraZeneca
- The drug has a slow off-rate (estimated half-life of 9 h for BACE1) , which may result in a prolonged reduction of Aβ.
- The phase I study, begun in 2014 and trial results demonstrated excellent safety, tolerability, and metabolic profiles in elderly healthy volunteers and in AD patients with mild cognitive impairment
- Like Merck's verubecestat, lanabecestat also strongly decreased the CSF Aβ level in the treated group.
- Phase II/III clinical trials recruited over 1400 participants and were planned to last up to 54 months with variable doses; they attempted to measure efficacy and safety in humans by analyzing results such as amyloid PET scans, CSF Aβ levels, and CSF amyloid inclusion.
- The clinical development program of this compound largely skipped Phase 2. Instead of running a medium-size Phase 2 followed by separate, larger confirmatory Phase 3 trials, the sponsors opted for a large, pivotal Phase 2/3 trial called AMARANTH.
- This trial compared AZD3293 to placebo given for two years in 2,202 patients who met NIA-AA criteria for MCI due to AD or mild AD.
- Each participant or his or her partner was required to report worsening in the past six months, and the participant's MMSE had to be above 21 at screening.
- In July 2016, a second Phase 3 trial started up. Called DAYBREAK-ALZ and conducted at 251 locations worldwide, it enrolled 1,899 patients with mild AD dementia as defined by an NIA-AA diagnosis of probable AD with a biomarker evidence of brain amyloid and an MMSE of 10 to 26.
- This four-arm trial compared two once-daily doses given for three years to two groups who start out on placebo for 18 months and then switch to either the low or high dose for the second half of the trial.
- On June 12, 2018, AMARANTH and DAYBREAK-ALZ were discontinued due to lack of efficacy determined at an interim futility analysis
- Lanabecestat reduced blood Aβ40 and Aβ42 levels by 70 to 80 percent in both trials.

Similar to that of verubecestat, the lesson learnt from this announcement is that the appearance of even mild symptoms may be too late in the disease continuum for a BACE1 inhibitor to be efficacious.

Atabecestat https://www.alzforum.org/therapeutics/atabecestat

- Daily administration of atabecestat 5–150 mg in healthy elderly and young participants for up to 14 days showed significant and consistent reduction of Aβ (up to 90% in the 90 mg cohort) in both plasma and CSF
- Although minor adverse effects such as headache and back pain were noted in the phase I trial, it was deemed safe enough to advance to phase II trials.
- a multicenter phase II trial, recruited 114 pre-dementia individuals to determine the tolerability and long-term safety of atabecestat, including double-blind treatment for 6 months.
- Atabecestat was found to successfully reduce both plasma and CSF levels of Aβ1-37, Aβ1-38, Aβ1-40, and Aβ1-42 in a dose-dependent fashion, while levels of sAPPα were conversely increased.
- In a separate trial called EARLY that was launched in 2015, participants are asymptomatic but at risk of developing Alzheimer's dementia and were intended to receive drug or placebo once daily for up to 4.5 years with continuous monitoring of cognitive scales.
- Unfortunately, observation of elevated liver enzymes in two patients led Janssen to announce the discontinuation of this trial on 17 May 2018.

Elenbecestat (E2609) https://www.alzforum.org/therapeutics/elenbecestat:

- Elenbecestat (E2609), originally developed by Eisai as a small-molecule inhibitor of BACE1, is currently in clinical trials co-developed with Biogen.
- A phase I trial reported that a single dose of 50 mg in 73 healthy participants (either gender, from age 30 to 85 years in six separate cohorts) was well-tolerated and safe.
- "A single oral ascending-dose study of 5–800 mg and a 14-day multiple oral ascending-dose study of 25–400 mg showed that elenbecestat could significantly reduce plasma or CSF Aβ levels by as much as 92%: plasma Aβ relative to baseline was 52% at 5 mg and 92% at 800 mg"
- In addition, by comparing different doses to placebo in mild cognitive impairment/prodromal patients or two doses in subjects with mild AD dementia, elenbecestat was found to delay clinical symptoms at the endpoint of the trial
- A phase IIa trial concluded that of elenbecestat 50 mg/day was safe and consistently reduced CSF Aβ by about 70%.
- Thus, a standard phase III trial called MISSION AD was initiated in November 2016 and MISSION AD2 was initiated in January 2017, enrolling a total of 1330 early AD subjects.
- Encouragingly, and unlike earlier mentioned discontinued trials, Biogen announced in June 2018 that the 18-month-long phase II study had revealed less decline in functional cognition in addition to a significant reduction in Aβ levels, quantified by amyloid PET imaging, in patients with mild to moderate form of AD

Data of Individual anti-inflammatory

- According to the neuroinflammation hypothesis underlying AD, there is a lower incidence of AD among users of chronic non-steroidal anti-inflammatory molecules (NSAIDs)
- Anti-inflammatory compounds, inhibiting COX activity, Naproxen and Celecoxib have been tested in clinical trials against AD.
- Naproxen, a non-selective COX inhibitor was administered (220 mg/twice day for two years) to 195 pre-symptomatic AD subjects (aged 55+) with a familial history of AD. The progression of the disease was evaluated with the Alzheimer's Progression Score (APS). Naproxen reduced the rate of the APS, though not significantly
- Celecoxib, a selective COX-2 inhibitor, was administered (200 mg/twice day for 2 years) in 677 pre-symptomatic subjects (70+) with at least one first-degree relative with AD. No improvement in the cognitive symptoms in the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT) in the AD patients compared to the placebo group was found.
- Furthermore, the specific TNF-α inhibitor, Etanercept, was evaluated in a small group of 41 AD patients (55+) with mild to severe AD (SMMSE score between 10 and 27), to test its anti-inflammatory effect and subsequent improvement of cognitive function.
    - The weekly 50 mg subcutaneous administration was well tolerated; however, after 24 weeks of treatment, Etanercept did not show significant beneficial effects in cognition, behavior, systemic cytokine levels or global function compared to the placebo-treated group
- The failure of this clinical trial involves many factors, including insulin resistance thus inhibiting specifically the TNF-α action may not be sufficient to counteract the inflammasome activity, and hence, to effectively prevent disease, perhaps due to the short period of time of assays.

Data of cholinesterase inhibitor

- These drugs have been approved for use in mild to moderate Alzheimer's disease. In Germany, three different cholinesterase inhibitors are currently available: donepezil, galantamine and rivastigmine. They are taken in the form of tablets. Rivastigmine is also available in a patch. Here the drug is absorbed into the body through the skin.
- **Galantamine appears to stimulate the release of acetylcholine and strengthen the way certain message-receiving nerve cells respond to it.**

Rivastigmine may block the activity of another enzyme involved in breaking down acetylcholine.

- In clinical trials of all three cholinesterase inhibitors, people taking the medications performed better on memory and thinking tests than those taking a placebo, or inactive substance
- Some of the people with Alzheimer's who regularly took one of these medications were able to remember things more easily.

Data of Memantine

- One clinical study showed that people taking memantine showed a small but statistically significant improvement in their mental function and ability to perform daily activities.
- But study participants with the lowest cognitive functioning showed no improvement on either daily activities or overall function.
- Another study randomly assigned participants to receive either 10 mg of memantine twice a day or a placebo in addition to donepezil (Aricept), a cholinesterase inhibitor.
- Those receiving memantine showed a statistically significant benefit in mental functioning and performing daily activities, while participants taking donepezil plus placebo continued to decline.

Data of Combination of memantine and donepezil:

- Namzaric® , a combination of donepezil and memantine, was approved by the FDA for the treatment of moderate-to-severe Alzheimer's in people who are taking donepezil hydrochloride 10 mg.
- Individuals taking Namzaric may see an improvement in cognition and overall mental function, and a temporary slowdown in the worsening of symptoms. However, there is no evidence that Namzaric prevents or slows the underlying disease process in patients with Alzheimer's disease.

Data of Combination therapies:

There are two DMTs currently in phase III trials that address two targets and represent valid combination therapies: ALZT-OPT1 and Gamunex (immune globulin intravenous (human), 10%; Grifols Therapeutics, Clayton, NC, USA).

- The ALZT-OPT1 trial, a combination regimen with cromolyn (anti-amyloid agent) and ibuprofen (anti-inflammatory agent), is enrolling patients with early AD who are either receiving or not receiving standard-of-care agents

Table II. Studies of combination therapy or add-on therapy for treating or preventing Alzheimer's disease (AD). Only studies that assessed the effects of treatment on cognitive performance, Clinical Global Impression of Change (CGIC) or measures of activities of daily living (ADL) and behaviour are included in this table

Combination therapies	Sample size and design	Outcome variables	Study duration	Dose schedule	Effects	Comments
Memantine, donepezil[67,68]	403 patients with moderate to severe AD; RCT, add-on design	SIB, ADCS-ADL	24wk	Donepezil at stable doses (5 or 10 mg/day) + memantine 20 mg/day (10mg bid titrated over a 4-wk period) or Donepezil at stable doses (5 or 10 mg/day) + placebo	At wk 24, patients treated with memantine plus donepezil showed significant improvement ($p < 0.001$) in cognitive function (SIB), compared with patients treated with donepezil plus placebo, and showed significantly less decline ($p = 0.028$) in daily function (ADCS-ADL) [2-way analysis of covariance]	Patients were receiving donepezil for the immediate preceding 3mo (and throughout the study period) and were randomised to receive memantine or placebo
Tacrine, estrogen[69]	343 female patients with AD (MMSE: 10–26); RCT; add-on design	ADAS-cog, CIBIC	30wk	Placebo or ERT + placebo or Tacrine 40–160 mg/day + placebo or Combination of ERT and tacrine 40–160 mg/day	ITT analysis (two sample t test, adjusted by baseline scores): ADAS-cog: $p = 0.01$ (ERT and tacrine vs tacrine alone) CIBIC: $p = 0.15$ (ERT and tacrine vs tacrine alone)	Tacrine treatment as the only randomisation/ nonrandomised ERT treatment, differences in age and education among ERT users
Rivastigmine, HRT[70]	117 menopausal women with AD (MMSE: 10–26); RCT; add-on design	ADAS-cog, IADL, MMSE, NPI	28wk	Rivastigmine (up to 12 mg/ day) + HRT or Rivastigmine (up to 12 mg/ day) + placebo	No significant changes in favour of HRT were noted on any efficacy parameters (ITT analysis of HRT vs placebo in menopausal women treated with rivastigmine, Wilcoxon test)	Patients were receiving rivastigmine and were randomised to receive HRT or placebo
α-Tocopherol, selegiline[28]	341 patients with moderate severity of AD (CDR: 2); RCT; combination design	Time to the occurrence of any of the following: death, institutionalisation, loss of ability to perform basic ADL, or severe dementia (defined as a CDR of 3)	2y	Selegiline 5mg bid (n = 87) or α-Tocopherol 1000IU bid (85) or Combination of selegiline 5mg bid and α-tocopherol 1000IU bid (85) or placebo (84)	In analyses that included the MMSE baseline score as a covariate, there were significant delays in the time to the primary outcome for the patients treated with selegiline ($p = 0.012$), α-tocopherol ($p = 0.001$) and combination therapy ($p = 0.049$), compared with the placebo group (Kaplan-Meier estimation)	Combination therapy with α-tocopherol plus selegiline did not provide an additional benefit compared with either treatment alone
Donepezil, tocopherol[71]	130 patients with AD (MMSE: 10–24); retrospective chart review; combination therapy	MMSE	3y	Donepezil 5 mg/day (at least) + tocopherol 1000 IU/day (at least)	Average cumulative change in MMSE at the 1-yr follow-up ($p = 0.0097$), at the 3-yr follow-up ($p = 0.0382$) [independent samples t test]	Retrospective chart review

Continued next page

- Cromolyn is a treatment for asthma approved by the US Food and Drug Administration (FDA) that bears structural similarity to other anti-amyloid agents and is likely to cross the blood-brain barrier
- Cromolyn reduced Aβ fibrilization and oligomerization *in vitro* and reduced $A\beta_{40}$ and $A\beta_{42}$ monomer concentrations in the mouse brain; oligomerization and fibrillation were unchanged *in vivo*. ALZT-OPT1 is a true combination trial in that the combination targets multiple disease pathways (amyloid and inflammation) and includes multiple methods of administration (intranasal inhaler for cromolyn and oral tablet for ibuprofen). ALZT-OPT1 is also an add-on study because it allows patients to continue standard-of-care treatments on stable doses."

Combination therapy approaches

Table II. Contd

Combination therapies	Sample size and design	Outcome variables	Study duration	Dose schedule	Effects	Comments
Selegiline and tacrine, physostigmine[72]	10 patients with AD; case series, crossover design, add-on therapy	ADAS-cog, MMSE	4wk	Tacrine + selegiline 5mg bid or physostigmine + selegiline 5mg bid; then Tacrine + placebo or physostigmine + placebo; or Tacrine + placebo or physostigmine + placebo; then Tacrine + selegiline 5mg bid or physostigmine + selegiline 5mg bid	ADAS-cog: p = 0.04 (Wilcoxon test), first period effect for selegiline compared with placebo; MMSE: p = 0.55 (nonsignificant Wilcoxon test)	Small number of patients; change scores were analysed for the comparison of the groups that first received selegiline with all groups that first received placebo
AChE inhibitor and α-lipoic acid[73]	9 patients with AD and related dementias; case series; open, uncontrolled study; add-on design	MMSE, ADAS-cog	337 ± 80 d	AChE inhibitor + α-lipoic acid 600mg	Stabilisation of cognitive functions (constant scores in MMSE and ADAS-cog) for at least 337 days	α-Lipoic acid was added to patients' existing standard treatment with AChE inhibitors; small sample size, short duration, lack of placebo control group, unblinded dosage
Donepezil and d-cycloserine[74]	5 patients with AD; case series; open uncontrolled study; add-on design	MMSE, ADAS-cog, IADL, CGIC	4wk	Long-term donepezil 5–10 mg/day treatment for at least 8 months + D-cycloserine 100 mg/day	No statistically significant effect found with any outcome measures examined at follow-up (MMSE p = 0.1, ADAS-cog p = 0.08; related sample t-test)	D-cycloserine was added to patients' existing treatment with donepezil; small sample size, short duration, lack of placebo control group, unblinded dosage
Pravastatin, tocopherol[75]	41 men and women with low-density lipoprotein cholesterol, cognitively intact, aged ≥70y; RCT, add-on therapy, crossover study	GHPQ, GDS, IADL, Digit Symbol Test, SDS	12mo	Pravastatin 20 mg/day for 6mo followed by pravastatin 20 mg/day + tocopherol 400 IU/day Tocopherol 400 IU/day + placebo for 6mo, followed by tocopherol 400 IU/day + pravastatin 20 mg/day	No significant changes occurred in any of the health-related quality of life or cognition measures after 6 or 12mo of therapy with pravastatin, tocopherol or their combination (assessed by nonparametric U-statistics)	No AD patients

AChE = acetylcholinesterase; **ADAS-cog** = Alzheimer's Disease Assessment Scale – cognitive subscale; **ADSC-ADL** = Alzheimer's Disease Cooperative Study-ADL Inventory; **bid** = twice daily; **CDR** = Clinical Dementia Rating; **CIBIC** = Clinician's Interview-Based Impression of Change; **ERT** = estrogen replacement therapy; **GDS** = Geriatric Depression scale; **GHPQ =** Global Health Perception Question; **HRT** = hormone replacement therapy; **IADL** = Instrumental Activities of Daily Living measured on the Assessment of Living Skills and Resources questionnaire; **ITT** = intention to treat; **IU** = international units; **MMSE** = Mini-Mental State Examination; **NPI** = Neuropsychiatric Inventory; **RCT** = randomised controlled clinical trial; **SDS** = Sleep Dysfunction Scale; **SIB** = Severe Impairment Battery.

Conclusion:

Given the complexity of AD, treatment of patients remains challenging. The currently approved treatments for AD are limited to cholinesterase inhibitors and memantine or the combination of these agents.

The high failure rate of the therapies in development for AD in large part comes from the complex pathologic causes of the disease, as well as our incomplete understanding of the relationships among the numerous pathways involved in development of AD and subsequent neurodegeneration, and the ineffectiveness of available agents..

Although single agent therapy has the advantage of simplicity, ensuring patient compliance and allowing straightforward identification of the source of adverse effect, monotherapy also has many limitations. AD is a product of multiple pathophysiological pathways. One drug blocking a single step in a complex pathogenic network often cannot block all crucial disease-propagating mechanisms.

Combining therapeutic agents may allow for lower doses of the individual agents, reducing costs and side effects. Innovative and adaptive clinical trial designs may also capture the potential evolution of therapeutic combinations over the long and complex course of disease progression, with one set of agents appropriate for preclinical AD, another for early-stage AD, and yet another for AD dementia.

Other non-therapeutic factors such as genetics, diet, lifestyle, health parameters like obesity, diabetes, high cholesterol, sleep patterns, etc, need to be studied in order to determine impact on the cause and progression of this disease. A few studies conducted in this area have shown the importance of maintaining proper health ie. normal cholesterol levels, average weight, blood glucose, adequate sleep, and daily exercise. Another area of importance is keeping your brain active. The experience of Alzheimer's is ultimately a result of losing synapses. Engaging in mentally stimulating activities increases your cognitive reserve because this creates an abundance of synaptic connections.

BACE inhibitors have been unsuccessful so far and one of the reasons why is because the appearance of even mild symptoms may be too late in the disease continuum for a BACE1 inhibitor to be efficacious. Hence, another step would be to identify individuals that are at risk of developing Alzheimer's. These would be people with a family history of Alzheimer's, those carrying the APOE4 gene, people with Cardiovascular diseases, high cholesterol, and diabetes. Having one or more of these risk factors increases the probability of one's development of Alzheimer's. Identifying individuals at risk can then

allow for preventative measures to be taken and the administering of preventative agents such as BACE inhibitors.

After completing my research, I have come to the conclusion that having clinical trials for treatments on high risk individuals started early, with a combination of agents like BACE inhibitors and amyloid antibodies, as well as cholinesterase inhibitors and memantine along with other combination drugs could identify a new therapeutic approach. The data gathered from my research proves that a combination of approaches that target more than one cause of AD such as neuro-inflammation, misfolded proteins, amyloid-beta plaques, and mitochondrial dysfunction will be most effective in the treatment of Alzheimer's Disease, thus proving my hypothesis correct.

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William D. Pratt Science Fair Planning Sheet

(this form is due by **January 28** and must be approved before beginning your project)

Name(s) & connect class: Ziya Bhayani Connect: Truss 8-1

Area of Study (chemistry, astronomy, etc.); Biology, Neuroscience/Neurology

Research based or experimental? (select one) **Research based**

Question or purpose of experiment; Will a combination therapy be more effective compared to single drug therapy in Alzheimer's Disease?

Hypothesis: A combination of approaches that target more than one cause of AD such as neuro-inflammation, misfolded proteins, amyloid-beta plaques, and mitochondrial dysfunction will be most effective in the treatment of Alzheimer's Disease.

Materials: Articles, published literature, interviews with specialists, books and other literature.

Proposed Procedure: 1. Better understanding of processes involved in the progression of AD like neuro-inflammation, misfolded proteins, amyloid-beta plaques, mitochondrial dysfunction, and other contributing elements of Alzheimer's disease as well as the types, stages, and complications.
2. Exploring inhibition of these processes from occurring at the onset.
3. Studying clinical trials that are based on the concept of combination therapy involving two or more drugs that target two different causes/processes.
4. Investigating the mechanism of the drugs that are currently being tested.
5. Deriving conclusions from the literature reviews. Searching for additional supportive information e.g.: facts/ diagnostics/ treatments using other strategies.
6. Proposing a possible combination of drugs that could be used and identifying possible areas that require further research.

Will you be experimenting on any plants or animals? (humans are animals) Yes or No

Why did you choose this topic? Alzheimer's disease is known to affect one in 10 people ages 65 and older. As per WHO, 50 million people aged 65 and older are living with Alzheimer's or other pathologies involving dementia worldwide. This number is expected to double by 2030. These statistics are estimated to rapidly increase as the human lifespan increases. Currently it can be neither prevented nor reversed or treated. This disease is difficult for the patient as well as their caregivers and families. It is intriguing to research how certain common diseases can be so complex and challenging. I would like to understand more about whether or not it is possible to treat this disease and not just the symptoms.

Who can you ask for advice or get help from on this project? Specialists in this field, mentors working on drug creation of AD,neuroscientists, professors from universities.

Science Fair coordinator signature _____
Date: January 26th 2021. Parent Acknowledgement Received:_____
*You may NOT proceed with your project until you have permission from the science fair coordinator and parental permission has been sent in by note or email.