Modified IPSC Tregs Combating CBI in Alzheimer's

Present Technology:

Current treatments for chronic brain inflammation (CBI) in Alzheimer's disease (AD) use anti-amyloid antibodies such as Donanemab and Lecanemab to remove Alpha Beta (Aβ) plaques, the main cause of CBI in AD. Donanemab and Lecanemab target Aβ plaques at different stages of its formation. Lecanemab binds to the early protein aggregates of Aβ plaques (soluble protofibrils) which prevents the formation of Aβ plaques in the first place. Lecanemab is administered through an intravenous (IV) infusion every 2 weeks for 18 months, and then every 4 weeks after 18 months have passed. Donanemab targets N-terminal pyroglutamates (amino acid formed from the N-terminal glutamine) in Aβ plaques, triggering the process of microglial clearance (the process in which microglia use phagocytosis to clean dead cells and debris in the brain). Donanemab is administered through an IV infusion every 4 weeks until the Aβ plaque is removed from the brain.

This method of treatment results in the effective removal of these plaques, slowing down the rate of cognitive and functional decline. However, the use of these antibodies contain risks. While in treatment, patients require regular MRI scans, costing significant time and money. Also, anti-amyloid antibodies have poor blood brain barrier (BBB, a semipermeable layer of cells that determines what goes in and out of the brain). According to the National Institutes of Health (NIH), only about 0.1%-0.3% of anti-amyloid antibodies given to a patient go through the BBB, making the treatment slow and ineffective.

The use of anti-amyloid antibodies also causes increased vascular permeability, resulting in Amyloid-Related Imaging Abnormalities (ARIAs). ARIAs is defined as the swelling (ARIA-E) or bleeding (ARIA-H) of the brain that can cause symptoms like visual disturbances,

strokes and seizures, and even death. While ARIAs can be treated, it takes a lot of money and requires the treatment of anti-amyloid antibodies to be stopped, making the use of Donanemab and Lecanemab ineffective and costly.

History:

In 1904 Alzheimer's Disease (AD) was discovered by Dr. Alois Alzheimer; he was able to do this through his patient Auguste Deter, who showed severe paranoia and memory loss. The autopsy after she had passed away showed many abnormalities with her brain, with things like plaques and other irregular things happening. From the discovery to the late 20th century it was commonly believed that Alzheimer's was something that just came with age and not the disease it actually was.

During the 1970s the battle to better understand Alzheimer's took large steps, and during this time they were able to disprove the common myth that AD was incurable. Even though they now knew that it was a myth they only comforted the patients as they passed. In 1987 the First AD drug trial began for the drug called Tacrine. This drug allowed the person to have improved cognitive function, but it did not help cure or fix it, only making the disease more bearable. In 1993 Tacrine became the first FDA approved AD medication. Three other AD cholinesterase inhibitors, which helps with nerves communication to each other, also helping with cognitive function, were approved in the following years.

During the 2000s scientists began to use brain scans, using things like MRI for scanning, to learn about Alzheimer's disease so we could more effectively fight against it, this also helped differentiate it from other similar diseases like Parkinson's by looking at things like an abnormal metabolism. Rivastigmine was also made in the 2000s to help fight against Alzheimer's, this drug works similarly to other drugs of the time just helping the cognitive function of the person

but not stopping the problem. The 2010s brought many acts and a lot more awareness about Alzheimer's and the first clinical trial for AD prevention was started. One of the largest of these acts being the National Alzheimer's Project Act, this act helped with improving care and doing more research about Alzheimer's in order to be able to fight the disease. During 2020-2023, a new drug called Lecanemab was made and it was able to slow Alzheimer's disease by around 27% instead of what the other drugs had been doing by just helping with cognitive function.

A large part of our plan on stopping the brain inflammation caused by AD is through T-cells. The first time T-cells were discovered was in the early 1960s by Jacques Miller when he discovered the Thymus, which is where most T cells migrate to. Later in 1970 a different type of T-cell was discovered, that being the cytotoxic T cells or killer T cells. Through the rest of the 1900s we learned many more things about T cells like their metabolism, immune checkpoints and the tolerance of them.

Future Technology:

Our technology firstly involves the extraction of red blood cells from the patient and the induction of the "Yamanaka factors." This would transition the red blood cells into iPSCs. iPSCs have the ability to differentiate into almost any type of cell. This ability would be utilized to create healthy Tregs for modification.

Next, these healthy Tregs would be edited to produce autocrine Il-2 when the pro-inflammatory TNF-α cytokine is detected at high levels by the TNFR1 and TNFR2 receptors. IL-2 is a growth factor for Tregs (meaning that it causes the Tregs to divide), but can normally only be collected outside of the cell. Activation of the IL-2 gene remedies this. This would be done by incorporating an independent synthetic signalling pathway into the Tregs' genomes. Synthetic signalling pathways are man-made mechanisms within the cell that trigger

certain responses when an output (usually a particular substance known as a “ligand”,) is detected by an extracellular receptor. One linker would be attached to the TNFR1 transmembrane and another to the TNFR2 transmembrane. Both linkers would include —from closest to the transmembrane to the farthest— one terminal of a split Tobacco Etch Virus protease (TEV,) a TEV cleavage site (TCS,) and one terminal of a split dCas9. When rejoined, the TEV protease has the ability to separate protein chains at the TCS site. dCas9 is a type of deactivated nuclease that has the ability to locate and move to a gene when given a specific RNA sequence (gRNA.) Only one of the linkers would have two Nuclear Localization Sequences (NLSes) and the VP64, p65, and Rta (VPR) transcriptional activators fused to the C terminal of the dCas9. The other would have a Nuclear Exporter Signals (NES) upstream of the TCS. VPR are powerful proteins that, when interacting with a gene, have the ability to overcome gene repression and activate that gene’s transcription. NLSes can be thought of as a “tag” that triggers movement into the nucleus. On the other hand, NES is the opposite, triggering movement out.

This set up makes it so that when TNF-α is detected on both the TNFR receptors, the TEV terminals combine and regain function. They then cleave the linker at the TCS sites, allowing the two terminals of the dCas9 to combine and also regain function. The NLS is then uncovered and triggers proteins to bring the dCas9 into the nucleus. The custom gRNA within the dCas9 brings it to the IL-2 production gene. The VPR then would activate the IL-2 gene and begin autocrine IL-2 production.

Because large amounts of inflammatory TNF-α cytokines signifies that the immune balance is shifted away from Tregs, activating a growth factor only when TNF-α is detected makes it that Tregs only rapidly divides to restore usual immune function. Restoring immune balance then allows Tregs to effectively ‘calm’ overactive immune cells, which creates the CBI.

However, this activation needs to be undone in order to avert chronic stimulation of the CD25 (the receptor that senses IL-2). One downstream effect of excessive IL-2 is the complete activation of AKT. This activation can act as a marker to indicate that IL-2 production is becoming extreme. Therefore, basing the deactivation of IL-2 on this event prevents any permanent cellular damage. This activation is characterized by the phosphorylation of AKT's Ser473 binding site. This phosphorylation can be utilized to trigger an artificial intracellular pathway made up of custom proteins —orthogonal of the larger cell and encoded within the genome— that would cause the dCas9-VPR transcription factor to be tagged with the Ubiquitin protein. This tagging results in the destruction of the dCas9-VPR.

These systems allow for efficient Treg adaptation to immune dysregulation, hence halting CBI.

Breakthroughs:

Certain breakthroughs must be made for this technology. For instance, the possibilities of cross talk (signalling pathway overlap) would need to be identified when designing the custom proteins. Otherwise, accidental pathway-triggering could dysregulate the Tregs. Cellular complexity makes this difficult. Additionally, iPSCs would have to be more stable if used. Current iPSCs often form multiple mutations, which can be challenging to detect. Finally, we would need to pin down how the Tregs would enter the brain. This is because the blood-brain-barrier (BBB) has evolved to disallow foreign entry. Additionally, existing methods are inefficient, such as intranasal administration, which is constrained by low volume capacity and high mucus obstruction.

IL-33 has been shown to increase Treg brain infiltration. Therefore, one possibility for allowing Treg entry into the brain in Alzheimer's is the simultaneous administration of IL-33 and

Tregs. Tests would be conducted on mice models, as they share 98% of our DNA. Mice with the same genetic lineages and lifestyle will be chosen. A 5xFAD model would be used to simulate Alzheimer's, which involves the integration of five APP mutations to quicken Aβ plaque formation in the mie. The mice will then be divided into an early Alzheimer's group (2 months of age,) moderate Alzheimer's group (5 months of age,) and a severe Alzheimer's group (9 months of age.) These groups would be subdivided into a group that receives IL-33, and one that doesn't. For this experiment, the modified Tregs will have a fluorescent protein gene integrated into their DNA.

Each group receives the same dose of Tregs, while the IL-33-receiving subgroups get the same dose of IL-33 in addition. After three days, flow cytometry will be performed on the dissected mice ipsilateral hemispheres to view how many of the fluorescent Tregs made it through the BBB. The results will be compared against one-another to view the effectiveness of IL-33 administration in different stages of Alzheimer's.

Design Process:

Brain information in Alzheimer's disease is a very complex in depth topic with many perspectives. To produce the best technology we looked at several different methods. One of those ideas was using nanobot technology to deliver crucial medicine to the brain to stop or decrease brain inflammation. This idea would incorporate CMT technology which mimics essential nutrients to the brain to pass the Blood Brain Barrier (BBB). We would also use novel inorganic materials to create the nanobots to enhance the durability of the bot while keeping its small size. Using an external megantic field system we can transport the nanobots to the brain. However the cost of these materials, the current feasibility of advanced nanobots, and the aggregation and Clumping of the bots led us to discard this idea

We then looked into the idea of using a medical tape solution to implement long term medicine into the body. We decided on using a series of microneedle-based patches placed around the body that could be controlled wirelessly to continually disperse the medication in a controlled manner. The medications would be anti-inflammatory medications such as Baricitinib, Naproxen, and Celecoxib all in patch form. The system would use an AI based system to control the distribution of the medicine and where to distribute it in the body. The AI would look at different statistics of the patient given by different home medical devices to appropriately give anti inflammatory drugs. However due the fact that there is no good anti-inflammatory medication currently in patch given form, the unpredictability and limitations with AI, the cost for patients to get at home medical devices, and the current limited at home medical devices for Alzheimer's patients lead us to look for a better solution.

The last idea we discarded was a lifestyle application. This app would help optimize lifestyle for Alzheimer's patients by providing an optimized diet, exercise and sleep. Better diet and lifestyle has been proven to lower inflammation according to a NIH article in 2023. This app takes advantage of that. The app would take all your full information provided by the patient and their doctor including previous conditions, Alzheimer's progression and more. This information would be given into an AI to process and identify how much nutrients they need to lower brain inflammation efficiently. There would also be a chatbot to ask questions about what you can and can't do with Alzheimer's. This idea, while very promising, had some major flaws. First of all, diet changes don't cut down the root cause of Alzheimer's . Secondly, while less reliant on AI than the patch solution, It can still be unreliable. Lastly, the vast amounts of personal information on the application leaves it vulnerable to hackers and leaks. For these reasons we decided to look for another solution

The final solution we came up with was iPSC derived Tregs. This method is far superior to nanotechnology currently because of its feasibility. iPSC technology is already in late clinical research, while nano tech is just starting to get into clinical trials. Though the current cost of iPSC technology is high, we are much closer to creating large-scale production of iPSCs making it far more cost effective than nanotechnology. According to an article by Meena Gnanasgkharan, around 8-20% of comprehensive analysis in medicine resulted in hallucinations. By not relying on Artificial Intelligence (AI), unlike the Patch solution and application, we can more reliably trust our system to do its job. With no separate technology needed at home, our customers will not bear the cost of such expensive items. This system does not put medical information on insecure platforms so there is no chance of theft of information. We also directly address the roots of Alzheimer's, making the impact more substantial. For those reasons we decided to continue with this path and develop the idea even more.

Consequences:

By making T regulatory cells (Treg) create their own IL-2 they become able to outcompete pro inflammatory cells in Alzheimer's, helping them regulate. Tregs will be able to produce anti-inflammatory cytokine at a higher efficiency with more IL-2 which dampens harmful inflammatory T cells helping remove Aβ plaque. Without a build up of Aβ plaque, Inflammation goes down and cognitive decline is slowed . According to a Science Direct article by J. Chandler et al., current methods reduce cognitive decline by 20%. With our method we hope to achieve 60-80% reduced cognitive decline. The newly powered Treg cells can also continue doing their other jobs such as preventing autoimmunity and maintaining tolerance to substances.

iPSC technology, while very promising, has many possible negative outcomes. One of the main concerns when dealing with iPSCs is tumorigenicity (the ability to form tumors). During the selected cell's reprogramming a large majority (~96% on average) will not completely finish this process and will remain undifferentiated in a small population of differentiated cells. Even with a small amount of contaminated cells a tumor could form after the cells have been implemented into the body. Most tumors become Teratoma tumors which are masses of different kinds of tissue cells, however they are mostly benign. In the unlikely cass that they are malignant they can cause significant harm to the body.

In our technology we use viral vectors in the CRISPR systems. However viral vectors, being modified dead viruses, can cause immune reactions as they see the vectors as foreign objects. This immune response can cause inflammation and when treated can leave the body more compromised to infections. Infections in Alzheimer's patients can cause severe brain inflammation counteracting what our solution tries to solve.

Another CRISPR related problem is off target editing. Using a complex RNA guide system the lentiviral CRISPR can accidentally edit off target locations due to the similar gene sequencing. Computers can predict these locations and correct them. However, a 2017 study by the colombia university found that over 100 larger deletions or insertions were not predicted and therefore could not be corrected. These mistakes can activate cancer genes, disabling tumor suppressors, causing cell deaths, triggering immune responses, and cause large chromosomal rearrangements.

Graft Vs host Disease, while mostly solved by using autologous iPSCs, can still occur during the reprogramming of the cell. During this phase new antigens can be expressed leading the body to view the cells as foreign and attack the cells causing immune rejection. According to

a NIH research paper, iPSCs are reported to be rejected around 5-20% of the time. Immune rejection can cause severe immune weakness, fatigue, more inflammation, and can make our solution ineffective. These risks are a severe limitation to our technology, but with many already finding new solutions we may soon be able to discard these possible consequences.

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