

PBT2 – evidence based unique mechanism to treat Alzheimer’s Disease

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For nearly 30 years the ‘amyloid hypothesis’ has been the dominant focus of R&D in Alzheimer’s Disease.

At its heart, the ‘amyloid hypothesis’ for Alzheimer’s Disease (AD) positions amyloid deposits in the brain, composed of various forms of *A-beta* peptide, as causally linked to the progression of the disease. These extracellular plaques became the first target for anti-amyloid therapy. The goal of any drug was to reduce amyloid (by analogy, reducing cholesterol reduces the risk of heart disease).

In recent years a number of drugs have been tested in the clinic to see if patients suffering from AD can be helped. Most of these drugs have been based on the amyloid hypothesis. Unfortunately all of the later stage trials (ie Phase III trials) of these drugs that have been reported to date have failed. Even where some of these drugs were both designed to, and have demonstrated a decrease in amyloid, patients showed no improvement in cognition. Naturally, some began to question whether the amyloid hypothesis was right or wrong. Certainly, the few drugs that are currently approved treatments for AD, that offer only temporary help with symptoms, are not based on the amyloid hypothesis.

In contrast, Prana’s Phase IIa trial with PBT2 trial was successful. Although, in a sense PBT2 is also based on the amyloid hypothesis, PBT2 embraces a very important difference. A difference which we believe is the difference between our success, in which we are very confident, and the failure of other high profile trials that have been reported. Unlike other strategies, we target the initial disease progression steps that result in *A-beta* becoming toxic, being the interaction with metals in the brain. We understand that tight controls on metal distribution and homeostasis fatigue with age, enabling toxic metal laden *A-beta* oligomers to form in synapses impairing neurotransmission. PBT2 targets these metals, restoring neuronal function to treat the disease.

Targeting Amyloid Reduction

If the build up of brain amyloid is the key causative factor in AD, therapeutic strategies to remove the amyloid from the brain or prevent its production by neurons should be effective.

Antibodies

Numerous anti-amyloid antibodies are in clinical development and are designed to reduce brain amyloid burden. The most advanced of these, Bapineuzumab, is purported to target a wide spectrum of forms of *A-beta* peptides and amyloid. In a recently published brain imaging study [1] using PET imaging technology and a radioactive tracer, ‘PiB’, that binds ‘fibrillar’ amyloid, (a pre-plaque insoluble aggregated form of *A-beta*), Bapineuzumab significantly decreased the levels of this PiB sensitive fibrillar form of *A-beta* over a 78 week period. Yet, no cognitive changes were seen (the researchers suggest that the number of patients in the study (28) was too small to

expect to see a significant effect on cognition). Similarly, in an earlier Phase IIb trial [2] over the same duration (78 weeks) no cognitive benefits were seen with Bapineuzemab. Subsequent stratification of the data in the Phase IIb did demonstrate however that cognitive benefits were observed in AD patients who were non-carriers for the ApoE(4) genetic trait (known as ApoE(4) negative). On this basis, Bapineuzumab has the potential to be helpful for about 40% of the AD patients that are ApoE(4) negative, if Bapineuzumab succeeds in Phase III trials. Another monoclonal antibody in Phase III development is Solanezumab. It also targets *A-beta*, although at a middle domain of the peptide unlike Bapineuzumab which targets the N-terminal end of the peptide. It is postulated that Solanezumab primarily draws *A-beta* peptide from the brain to the periphery as suggested by 52 patient Phase II trial where both CSF and plasma levels of *A-beta* rose over 12 weeks. The study did not report any positive cognition findings. An alternative approach has been to investigate the effect of directly administering human plasma that contains native *A-beta* antibodies. Two intriguing pilot Phase II studies have been recently published using intravenous immunoglobulin (IVIg). In an 18 month open label dose ranging study with 8 patients [3], CSF levels of *A-beta*(40) peptide decreased by approximately 20% after 6 months and following a washout period – again at 18 months, levels of *A-beta*(42) peptide decreased by approximately 50% at 6 months and 40% at 18 months. Clinical cognitive assessment of the patients using the Mini Mental State Examination (MMSE) indicated that over the 18 months MMSE scores were maintained at close to the baseline value of 23.5 with a peak improvement to 26 at 6 months following the washout period, MMSE values fell to 23.9 and after a further 9 months of treatment the average MMSE score was 24. In recently announced results of another 18 month study which tracked 14 patients with uninterrupted IVIg treatment, both functional and cognitive measures improved. Using the Alzheimer’s Disease Cooperative Study-Clinical Global Impression of Change rating scale (ADCS-CGIC) a significant change of 1.36 points over placebo was observed and on the cognitive assessment using the Alzheimer’s Disease Assessment Scale-Cognitive Subscale (ADAS-cog) there was a significant 9.15 point improvement relative to placebo. The interesting mechanistic question is whether the cognitive/functional improvements observed, if reproduced in larger trials, was due to the native *A-beta* antibodies which effected a change in CSF *A-beta* levels as the amyloid hypothesis would suggest, or whether the non *A-beta* antibodies also contained in IVIg somehow benefitted neuronal health and function in a manner that is *A-beta* independent.

Secretase Inhibitors

Another strategy to decrease the overall amyloid burden in the brain is to inhibit the enzymes that are responsible for the generation of the *A-beta* peptide from its precursor protein APP; beta- and gamma-secretase. These are generally referred to as Secretase Inhibitors or Secretase Modulators. The first example of this class of agent to complete Phase III clinical testing was Flurizan. This ‘Selective Amyloid beta-42 Lowering Agent’ was designed to modulate gamma-secretase to selectively prevent the production of *A-beta*-42, thought to be the type of *A-beta* with the greatest tendency to aggregate and form plaques. However, the drug failed to meet any of its cognitive or functional endpoints and Flurizan’s development was stopped.

Another agent targeting gamma-secretase is Semagacestat, which is the most advanced gamma secretase Phase III agent at this time. Semagacestat has shown that it can potently drop plasma *A-beta* levels by approx. 60% within hours of administration [4]. However, there were no changes in CSF *A-beta* levels, nor were there any cognitive improvements detected in this short term (14 week) Phase II study [5].

The field awaits the results of the Phase III trials of this agent along with the anti-amyloid antibodies; Bapineuzumab, Solaneuzumab, and the pooled anti-A β antibody strategy comprising IVIG Gammaguard. Will these results be further ‘tests’ of the amyloid hypothesis?

Targeting Amyloid Aggregation

Another strategy has been to block the downstream consolidation of soluble *A-beta* oligomers into insoluble aggregates and plaques. Alzhemed is an agent that had shown evidence of decreasing plaque levels in transgenic AD mice, yet this ‘anti-aggregation’ agent failed to demonstrate cognitive or clinical benefits to patients in Phase III [6].

Currently in Phase II is the anti-aggregation compound ELND005. This compound binds to *A-beta* oligomers and prevents them from assembling into very large aggregates and fibrils. This 18 month trial has been significantly modified for safety reasons, and is now a single dose trial, with the two top doses of the initial three doses being withdrawn.

Targeting outside the Amyloid Hypothesis

Dimebon is an antihistamine purported to improve mitochondrial activity. Mechanistic data were never published to understand if this agent had any anti-amyloid activity or to explain how the action of Dimebon could translate into clinical effectiveness. While very promising results from a Phase II study conducted in Russia were published, the drug recently was reported to have failed the cognitive and efficacy endpoints of a Phase III trial. The future of Dimebon has not yet been revealed, but analysts expect its development for AD to be stopped.

Targeting the Real Culprit – Toxic ‘A-beta Oligomers’

Is reducing the amyloid burden the correct therapeutic strategy to address within the amyloid hypothesis? How do we reconcile that many adults have high amyloid burden as imaged by PiB yet remain asymptomatic? More importantly, is it not smarter to selectively target the minimal toxic unit of *A-beta*, the small soluble oligomers that form in the synapse and have been shown by many workers to impair synaptic function? This fraction of *A-beta* constitutes as little as 1% of amyloid forms in the brain [7], yet there is strong published evidence that this small pool significantly impairs neurotransmission at the ‘glutamatergic’ synapse (ie synapses relying on glutamate as the neurotransmitter, the most relevant in AD) [8].

Consistent with these findings is the hypothesis by Prana scientists that it is the interaction between *A-beta* with metals in the synapse, such as Zinc and Copper, which leads to the formation of small toxic oligomers of *A-beta* that inhibit neurotransmission.

Administration with Prana’s lead MPAC, PBT2, prevents the interaction of synaptic Zinc and Copper with *A-beta* to prevent the *A-beta* from becoming toxic. Published work [9] demonstrates that this strategy results in restoring normal neurotransmission to synapses, otherwise impaired by *A-beta* oligomers, leading to rapid cognitive improvements in transgenic animal models.

PBT2 is able to achieve this by ‘disarming’ the *A-beta* monomers and oligomers of synaptic metals, to achieve two beneficial outcomes:

- (i) Prevention of toxic metal-laden oligomer formation and resulting synaptic toxicity
- (ii) Return of critical synaptic metals, otherwise bound to oligomers, to neurons, facilitating normal neurotransmission [10].

Prana scientists believe that these established capabilities of PBT2 were critical in causing significant improvement in cognitive Executive Function in a twelve week Phase IIa trial with PBT2. Prana’s position is that the answer to treating AD does not lie in disputing the relevance of amyloid to AD, but rather to adopt a strategy which will prevent the initial seeding event in the path to amyloid toxicity; the interaction between synaptic metals and *A-beta*. For this approach to be right, we need to ask “why then is AD predominantly a disease of the aged”? Prana scientists have investigated this and shown that with increasing age, the normal tight regulation of metal transport and localization in the brain weakens. Notably, that the normal mechanisms of pumping Zinc and Copper back into neurons (after an electrical signal passes from one neuron) become fatigued resulting in a buildup of metals in the synaptic cleft (the narrow space between communicating neurons). Accordingly, *A-beta* that is normally released into the same synaptic space, has a high chance of interacting with the metals to form metal-laden oligomers which are toxic to neurons, leading to cognitive impairment.

Potential concerns if the therapeutic strategy is not specific for the toxic oligomers of A-beta

Debulking the brain of amyloid as a therapeutic strategy also brings its own challenges for drug development. What level of amyloid decrease is tolerable without off-target safety issues? For example, in both the Phase II trial and recent imaging study with Bapineuzumab, some patients treated with the antibody developed cerebral vasogenic oedema – resulting in small hemorrhages and bleeding in the brain. A consequence of the antibody being relatively unselective to the type, or site of amyloid, is that vascular amyloid was targeted. It is also possible that there will be other consequences with long term administration given that the antibody can also ‘see’ the precursor protein APP which has various other roles in the body. As for the secretases, they have numerous activities other than simply producing the *A-beta* peptide (eg managing Notch, a protein critical for cell fate and development): Will it be possible to find the right dosage and form of a drug that will not interfere with the other non-amyloid functions of the secretases?

Moreover, new evidence suggests that non toxic forms of *A-beta* could have normal roles in the body, such as recently identified antimicrobial activity in the brain [11]. Indeed, it has been reported that a certain amount of *A-beta* is now considered important for optimal synaptic function [12]. Therefore, there are numerous challenges ahead for therapeutic strategies that seek to prevent *A-beta* production or unselectively remove *A-beta* from the brain.

In Summary

Prana is a hypothesis driven company. The science and mechanistic rationale for how PBT2 may translate into clinical benefits for AD sufferers has been published and presented under top peer review. *The mechanistic rationale for PBT2 has not been diminished by the mixed results of other ‘anti-amyloid’ strategies.* Rather, it sits squarely as a therapeutic strategy that tackles the genesis of *A-beta* induced toxicity in the brain – by targeting the basic interaction between metals and *A-beta* in the synapse.

Glossary

Abeta / Beta amyloid - is an amyloid derived from a larger precursor protein and is a component of the neurofibrillary tangles and plaques characteristic of Alzheimer's disease.

ADAS-cog is short for 'Alzheimer's Disease Assessment Scale'. ADAS was designed to measure the severity of the most important symptoms of Alzheimer's disease (AD). Its subscale 'ADAS-cog' is the most popular cognitive testing instrument and consists of 11 tasks measuring the disturbances of memory, language, praxis, attention and other cognitive abilities which are often referred to as the core symptoms of AD.

ADCS-CGIC is a comprehensive global assessment of the patient by the physician incorporating caregiver input.

Amyloid is a waxy translucent substance consisting of protein in combination with polysaccharides that is deposited in some animal organs and tissues under abnormal conditions (as Alzheimer's disease).

Amyloid plaque is a build up of amyloid protein and a primary hallmark of Alzheimer's disease.

Antihistamine is a drug that inhibits the actions of histamine. Histamine causes dilatation of capillaries, contraction of smooth muscle, and stimulation of gastric acid secretion.

APP - amyloid precursor protein is a gene, when mutated, which causes an abnormal form of the amyloid protein to be produced.

ApoE is the abbreviation for apolipoprotein E - a gene that codes for a protein in lipoproteins (complexes of fat + protein) that are normal constituents of blood plasma -- namely, chylomicrons, HDL (high density lipoprotein), LDL (low density lipoprotein), and VLDL (very low density lipoprotein).

Beta-secretase is an enzyme that catalyses the splitting of interior peptide bonds in a protein. Beta-secretase acts by trimming off a protein protruding from a brain cell. This small snip is thought to be the first step in the buildup of microscopic balls of debris known as amyloid that are toxic to brain cells.

Blood-brain barrier is the selective barrier that controls the entry of substances from the blood into the brain.

Copper is a [chemical element](#) that is essential in all plants and animals.

CSF - cerebrospinal fluid is a watery fluid, continuously produced and absorbed, which flows in the ventricles (cavities) within the brain and around the surface of the brain and spinal cord.

Cognition is the process of knowing and the process of being aware.

Dementia is the mental deterioration of organic or functional origin.

Gamma secretase is an enzyme partly responsible for plaque buildup in the brain characteristic of Alzheimer's.

Glutamatergic is a term that pertains to the action of glutamate or to neural or metabolic pathways in which it functions as a transmitter.

Immunoglobulin is a protein produced by plasma cells and lymphocytes and characteristic of these types of cells. Immunoglobulins play an essential role in the body's immune system. They attach to foreign substances, such as bacteria, and assist in destroying them.

Inflammation is a basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. Inflammation is now recognized as a type of nonspecific immune response.

MMSE is the 'Mini-Mental State Examination' which is a standard mental status exam routinely used to measure a person's basic cognitive skills, such as short-term memory, long-term memory, orientation, writing and language. The MMSE concentrates on the cognitive aspects of mental functioning, excluding questions about the patient's mood or such abnormal experiences as dissociation.

Neurodegenerative is a term that related to or characterized by degeneration of nervous tissue.

Neuron is one of the cells that constitute nervous tissue, that have the property of transmitting and receiving nervous impulses.

Neurotransmitter is a substance (as norepinephrine or acetylcholine) that transmits nerve impulse from one cell to another across a synapse.

Neurofibrillary tangles is a fine fiber found in cytoplasm signalling an abnormality of the hippocampus and neurons of the cerebral cortex that occurs especially in Alzheimer's disease.

Oligomer consists of a few [monomer](#) units, such as [dimers](#), [trimers](#) and [tetramers](#).

PIB is the 'Pittsburgh Compound B' ligand that binds to β -amyloid in extracellular plaques and vessel walls, which is used as biomarker for amyloid in Alzheimer's disease.

Peptide is a small molecular fragments that come from two or more amino acids by combining the amino group of one acid to the carboxyl group of another. They are obtained by partial hydrolysis of proteins.

Redox is a reversible chemical reaction in which one reaction is an oxidation reaction and the reverse a reduction.

Secretase is the enzyme involved in cutting amyloid into the shorter beta-amyloid form.

Synapse is the point of connection usually between two nerve cells. More specifically, a specialized junction at which a nerve cell (a neuron) communicates with a target cell.

Tau is a protein which channels chemical messages inside nerve cells.

Transgenic mice are an experimental strain of mice that has been genetically engineered in the laboratory such that a specific gene is added to the DNA of the mouse.

Vasogenic oedema is an accumulation of water and sodium in the brain that results from the breakdown of the blood-brain barrier and is seen with brain tumours, abscesses, [haemorrhage](#) and [contusion](#). The increased [permeability](#) of the capillary endothelium leads to extravasation of fluid, plasma proteins, mainly albumin.

Zinc is a mineral that is essential for proper nutrition.

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