



## Genesis of Alzheimer's - Brain or Blood: Are we looking at the Right Drug Targets?

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### ABSTRACT

The number of elderly dependents is believed to increase globally and reach 277 million by 2050. Almost half of such dependents are likely to be affected with some form of dementia; Alzheimer's being the most common. Generally accepted hypothesis about Alzheimer's disease (AD) is secretase enzyme dependent formation and aggregation of amyloid beta ( $A\beta$  1-42). In normal condition,  $A\beta$  does not cause formation of plaques in brain. Several factors including enzymatic degradation, receptors (LRP-1, sLRP-1 and RAGE), carrier proteins (albumin, apoE, apoJ, TTR and 2m), endothelial cell of blood brain barrier (BBB) and epithelial cells of Choroid Plexus (CP) help in clearance of  $A\beta$ . The brain of AD patients with a leaky BBB seems to be associated with apoE4 allele, suggesting that the BBB dysfunction along with alteration in  $A\beta$  degrading enzyme activity, changes in receptor expression & hormone imbalance (especially stress related hormones) might have a critical role in AD. Report for racial and ethnic disparities based on mediclaims filed suggest that maximum tendency to develop AD is shown by Hispanics, followed by African Americans, Whites, Native Americans, and Asians. The focus of this review is on an alternative hypothesis that possibly, the genesis of AD happens much before the appearance of clinical symptoms, and that the components delivered by blood, and alteration in BBB may play a vital role in the initiation of pathogenesis. Several factors like immunoglobulin's, type-II diabetes, formation of  $A\beta$  fibrils and tangles in pancreas, contributions of platelets to circulating levels of  $A\beta$  and neurological disorders associated with the patients of cardiovascular diseases are suggestive that the pathogenesis of AD may be checked from other, non-neuronal organs. These factors/locations can also be potential drug targets. This alternate approach can provide the necessary impetus to future research for treating AD.

**Keywords:** Dementia, Amyloid beta, Cerebral Amyloid Angiopathy, blood brain barrier, chloride plexus.

### INTRODUCTION

Dementia is an overall term for clinical conditions characterized by a decline in memory or other cognition that affects a person's ability to perform everyday activities. Dementia is caused due to damage of neurons in brain, resulting in abnormal functions and neuronal death. This leads to changes in one's memory, behavior and ability to think. Different types of dementia are vascular dementia, Dementia with Lewy Bodies (DLB), Front Temporal Lobar Degeneration (FTLD), mixed dementia, Parkinson's Disease (PD) dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus and Alzheimer's disease (AD). AD is the most common form of dementia, which accounts estimated 60-80% cases.

A century ago, German physician, Alois Alzheimer first described the classical symptoms of AD: presence of extracellular deposits of a substance and tangles in brain & blood vessels of his patient Auguste Deter, for the disease that is now associated with his name, Alzheimer's disease<sup>1</sup>.

In Alzheimer's disease, the damage and death of neurons eventually impair one's ability to carry out basic bodily functions such as walking and swallowing. People in the final stages of the disease are bed-bound and require

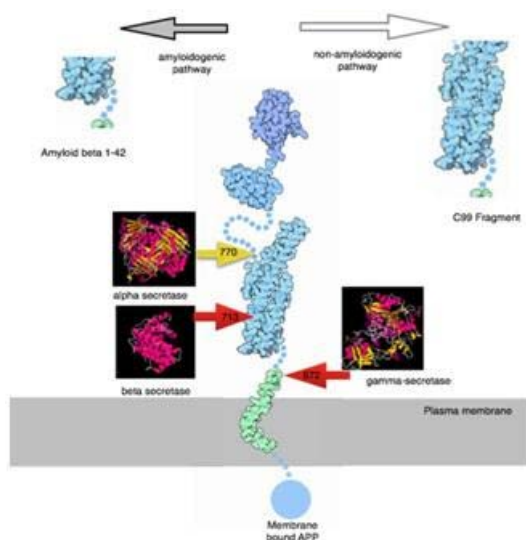
around-the-clock care. Alzheimer's disease is ultimately fatal<sup>2</sup>.

AD is not a normal part of ageing. The cases of dementia increased around 28 millions in last 4 years and are escalating day-by-day, increasing with the rate of 7.7 million new cases every year worldwide<sup>3</sup>. WHO report support the Alzheimer's disease International (London, United Kingdom) statistics of 2014, which reported that there are nearly 36 million patients of Alzheimer's or related dementia worldwide. The report further suggested that only one in four people with AD have been diagnosed so far<sup>4</sup>. In USA alone there is estimated 5.2 million people with AD. A new case will arise in every 67 seconds in USA which suggested that more than 13 million Americans will have the disease by 2050. Currently AD is the sixth leading cause of death in USA and one third of old age people died due to AD last year in USA<sup>5</sup>. Alzheimer's Related Disorder Society of India's (ARDSI) estimated that the number of people with dementia in India is 3.69 million and the number is set to double in next 20 years. ARDSI report projects that there would be around 14.2 million patients with AD in India in 2050 and this number would be higher than number of projections in UK and US in future<sup>6</sup>. The increasing number of patients & the huge cost of the disease will challenge health systems in future. The costs are



estimated at US\$ 604 billion per year at present and are set to increase quickly as the prevalence of AD proliferates. The high projections and extent of dementia/AD is now a matter of concern for all countries in the world. Many governments, public and supportive agencies are continuously monitoring the situation & patients of AD at international, national and regional levels & are increasing efforts for its cure. AD is now included in the public health agenda of many countries<sup>7</sup>.

Alzheimer's and other dementia are the top cause for disabilities in later life. There are several other possible causes of AD besides amyloid plaques formed by A $\beta$  like intracellular neurofibrillary tangles of tau protein, mutation in presenilins gene, Cerebral Amyloid Angiopathy (CAA), formation of apolipoproteins, appearance of advanced glycation end products, neuroinflammation, hormonal imbalance, stress in midlife, neuronal dysfunction and ultimately neuron death. The most prevalent hypothesis of AD is that it occurs because of secretase enzymes dependent altered cleavage of Amyloid Precursor Protein (APP)<sup>8</sup> which is located in the cell membrane of neurons hypothalamus. Amyloid precursor protein is a membrane bound protein. Under normal conditions,  $\alpha$  secretase enzyme cleaves APP at a site (amino acid position number 770), which yields a peptide P3 so that prevents formation of the  $\beta$ -amyloid peptide, and this pathway is called non-amyloidogenic (fig-1). However, activity of  $\alpha$  secretase is overshadowed somehow by the activity of  $\beta$  and  $\gamma$  secretase. These enzymes cleave APP into two specific positions (amino acid position 713 and 672 respectively) that generate a peptide called Amyloid Beta (A $\beta$ 42) (Fig-1). Overproduction of A $\beta$  peptides, as well as the failure of its degradation by enzymes, such as neprilysin and insulin degrading enzyme lead to A $\beta$  oligomerization and aggregation over time outside cell membrane of neurons ultimately leading to senile plaques formation that are the main neuropathological features of AD.



**Figure 1:** Processing of APP protein with beta and gamma secretase and production of Amyloid beta peptide

through amyloidogenic pathway. The production of C99 fragments through cleavage by alpha and gamma secretase enzymes on position number 770 and 672 amino acid respectively leads to non-amyloidogenic pathway.

Brain receives a profound blood supply and there is an intricate network of arteries and veins. The blood-brain-barrier (BBB) is able to protect the CNS from immune activation but it becomes leaky sometimes, for example during inflammation, which renders the brain vulnerable to infections. The leaky blood-brain-barrier in AD patients suggested that BBB dysfunction might contribute to the onset and/progression of AD.

Several groups of researchers are trying various techniques & methodology and aiming at different targets. However a miracle drug is still an illusion. The focus of this review is on an alternate hypothesis that possibly, the genesis of AD happens much before the appearance of symptoms, and the components delivered by blood, and alternation in BBB may play a vital role in the initiation of pathogenesis. Several factors like immunoglobulin, type 2 diabetes, formation of A $\beta$  fibrils and tangles in pancreas, contributions of platelets to circulating levels of A $\beta$  and neurological disorders associated with the patients of cardiovascular diseases are suggestive that the pathogenesis of AD may be checked from other, non-neuronal organs. These factors/locations can also be potential drug targets. This alternate approach can provide the necessary impetus to future research for treating AD.

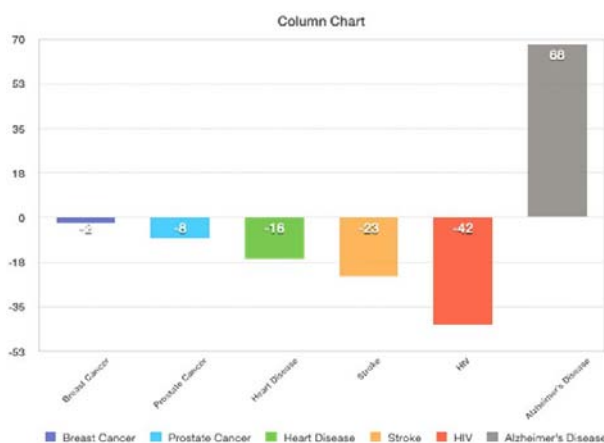
### The Economics of Dementia

President Barack Obama, United States of America in 2011 said in his proclamation, "...alzheimer's disease burdens an increasing number of our nation's elders and their families, and it is essential that we Confront the Challenge it poses to our public health..." His words highlighted the urgency on control and treatment of AD.

The number of older dependents is believed to increase globally from 101 million in 2010 to 277 million by 2050. Almost half of such dependents are likely to be affected with some form of dementia. In the present time, caring and treating patients with dementia is imposing staggering constraints on health systems world over. The cost of dementia makes a high financial impact upon families, governments, and their national and social care systems. According to the WHO, treating and caring for people with dementia currently costs the world more than US\$ 604 billion per year. This includes the cost of providing health and social care as well the reduction or loss of income of people with dementia and their caregivers. The 89% of total global societal economic cost (US\$ 604 billion) is incurred in high-income countries. The highest cost proportion for dementia is in USA and then Western Europe.

From 2000 to 2010, the percentage of deaths in the U.S. from most common disabilities like cancer, HIV/AIDs and

cardiovascular diseases declined, some sharply, while deaths of people with Alzheimer's skyrocketed (Fig 2). The seriousness of the patients suffering from AD is the total death caused last few years. CDC reported that in USA 83494 deaths occurred due to AD in 2010<sup>9</sup>. The CDC considers a person to have died from AD if the death certificate lists AD as the underlying cause of death, defined by the WHO as "the disease or injury, which initiated the train of events leading directly to death"<sup>10</sup>. Severe dementia frequently causes other complications also such as immobility, swallowing disorders, and malnutrition that can cause death<sup>9</sup>. One such condition is pneumonia, which has been found in several studies to be the most commonly identified cause of death among elderly people with AD and other dementias<sup>11,12</sup>. Hence Alzheimer's disease is a contributing cause of death for more Americans than indicated by CDC data (83494) because the CDC data do not count the people who died because of complications arising due to AD.



**Figure 2:** Percentage Changes in Selected Causes of Death (All Ages) Between 2000 and 2010

**Source of the data:** Alzheimer's association report (2014) Alzheimer's disease facts and figures, Alzheimer's & dementia, Volume 10, Issue 2.

Another report contradicting CDC report is of Chicago Health and Aging Project (CHAP). CHAP reported that, estimated 600,000 people died with AD in 2010, meaning they died after developing AD. Based on CHAP data, an estimated 700,000 people in the United States age 65 or older died with Alzheimer's in 2014<sup>13</sup>. Countries like USA and others keep detailed record of AD & they are already worried about the situation there. In other countries besides USA like the developing countries, there is neither any data recorded by the authorities nor big budget for regular health checkups. So the challenges faced by the health agencies in these countries can only be imagined.

For assessing the economic impact researches use two different methods. One method assess impact on paid healthcare only and other also considered value on unpaid care. These methods estimate that per-person cost of dementia ranged from \$56,290 or \$41,689 per person in USA.

Worldwide, the estimated annual cost of dementia was US\$ 604 billion (1.01% of world GDP) for the year 2010<sup>14</sup>, an increase by 43% of the 2009 estimate (US\$ 421.6 billion)<sup>15</sup> and almost double 92% of estimate for the year 2005 (US\$ 315.4 billion)<sup>16</sup>. Worldwide the number of people with dementia is expected to double over the next twenty years (35.6 million in 2010 to 65.7 million by 2030)<sup>17</sup>; just this increase would push the cost by 85% in 2030<sup>16</sup>.

A *New England Journal of Medicine* report last spring 2014 reported that Alzheimer's is the most expensive malady in the U.S. The monetary cost of dementia in the United States ranges from \$157 billion to \$215 billion annually, making the disease more costly to the nation than either heart disease or cancer (RAND Corporation study). The total cost evaluation of 17.5 billion hours of unpaid care provided to AD patients in 2012 and 17.7 billion in 2014 valued over 220.2 billion US\$<sup>18,5</sup>. It is nearly eight times the total revenue of McDonald's in 2012 and half of the net value of Wal-Mart sales. The future projections of Alzheimer's association USA suggest the expenditure on AD will be 1.2 trillions US\$ per year by 2050.

In different selected divisions of the world people paid a lot on dementia and AD. United Kingdom lost around £23.0 billion for the year 2008. Long term institutional care and informal care constituted nearly 95% of these costs. The annual cost per case of dementia was estimated to be £27,647, which was much more than the median salary in UK (£24,700) and was several times greater than that for Cancer (£5,999), Stroke (£4,770) or Heart disease (£3,455). The last studies reported in Canada has shown total economic burden due to dementia was estimated to be C\$ 15 billion for 2008 and expected 10-fold increase in 30 years. Australia shelled out 6.6 billion Au\$ on dementia in 2002, while Korea spend more than 2.4 billion US\$<sup>19</sup>. The total cost of illness for whole of Europe was estimated to be €177.2 billion of which €96.6 (55.0%) billion was due to informal care. Only study conducted in India was in Chennai and it stated that the risk for mortality was 2.3 times more for older people who received a diagnosis of dementia at the baseline survey. With an estimated 3.7 million people with dementia in 2010, the calculated total societal cost of dementia for India was estimated to be US\$ 3.415 billion (INR 147 billion). While informal care is more than half the total cost (56%, INR 88.9 billion), nearly one-thirds (29%) of the total cost is direct medical cost (INR 46.8 billion). The total cost per person with Dementia is US\$ 925 (INR 43,285) in India<sup>14</sup>. The pilot studies and the economic data undertaken on very few people (179 patients in 2 centers only) for AD study in India<sup>14</sup>. There is a basic need for good quality economic research and operational research, particularly in the Indian context. The diverse landscape of India prevented in estimating uniform average costs. The huge urban – rural divide, the ongoing process of rurbanization (urbanization of rural areas) and globalisation pose methodological challenges

in cost estimation. Like several low and middle-income countries, economic analysis of a disease & health situation is quite limited in the Indian subcontinent. With a lower priority for research, it is not surprising that ongoing and available research contributes little to economic analyses.

### Hypothesis of AD with Knowing Facts

Development of A $\beta$  senile plaques and tau tangles leading to main clinical/pathological symptoms of AD patients. But it may take several years to develop the classical symptoms. While the disease initiate and proliferate might start much early. The process of onset might be initiated through various different process in different other organs of body without any short-term characterization and symptoms. The discovery of the factors related to the progression of AD and its correlation with other organs of body and transportation to brain raises strong question about the genesis of AD.

The well-established fact from centuries is the oxygen transportation to the brain through blood. Growing evidences suggest that the health of the brain is also closely linked to the overall health of the heart and blood vessels. The brain is nourished by richest network of blood vessels and similarly healthy heart ensures enough blood supply through healthy blood vessels for its normal functions. For many years now, investigators are trying to find out the reason for occurrence and production of A $\beta$  peptides in other body organs besides brain. Decades before it was found that the APP protein along with the secretases enzymes was also present in other non-neuronal organs like pancreas, liver, blood cells (mainly in platelets), and other non neuronal tissues. In patients with AD, focal and diffuse ischaemic abnormalities of the cerebral white matter can be demonstrated neuropathologically and neuroradiologically. Many factors that increase the risk of cardiovascular disease are also associated with a higher risk of developing AD and other dementias. These factors include smoking,<sup>20-22</sup> obesity (especially in midlife),<sup>23-29</sup> diabetes<sup>22,30-34</sup> high cholesterol in midlife<sup>25,35</sup> and hypertension in midlife.<sup>25,28,36-38</sup> Hence we are hypothesizing that the alteration of blood and constituent, parameters and factors mentioned above like stress of present life-style of current time, initiate events that might leads to initiation of AD much earlier but diagnosis occurs at a much later stage. Some of the factors that play important role in AD and can be interesting candidates for drug targeting are:

### Receptors, Factors And Blood Brain Barrier In AD

A $\beta$  is naturally formed in our body but its concentration is rigorously regulated by its rate of production from the APP and its metabolic clearance. Mammalian brain is separated from blood by the BBB. Tight junctions are present between the endothelial cells of the capillaries that perfuse the brain parenchyma from the BBB (Fig-3). There are no effective barriers to diffusion of molecules between brain interstitial fluid (ISF) and Cerebrospinal

fluid (CSF). While the vascular barriers restrict the transport of polar solutes, rapid transport of essential hydrophobic nutrients, such as glucose and amino acids, and peptides and proteins involves specific transporter systems and/or receptor-mediated transport, respectively. A $\beta$  is a small peptide that can move across BBB through receptor-mediated transport only. The most common isoforms of A $\beta$  are A $\beta$ 40 and A $\beta$ 42. A $\beta$  peptides are also produced by many cells and circulate in plasma, CSF and brain ISF mainly bound to chaperone molecules in equilibrium with a small free unbound A $\beta$  fraction. A $\beta$ 40 levels are greater than that of A $\beta$ 42 in normal human CSF and plasma by about 10- and 1.5-fold, respectively<sup>39,40</sup>.

It has been shown in an *in-vitro* study that in CSF, apolipoprotein E (apoE), apolipoprotein J (apoJ), transthyretin (TTR), and  $\alpha$ 2- macroglobulin ( $\alpha$ 2M) can bind with A $\beta$ . Binding of these proteins to A $\beta$  influence its clearance across BBB, its metabolism and its aggregation in brain.<sup>41,42</sup> However, besides all these binding/carrier proteins, the low-density lipoprotein receptor related protein-1 (LRP-1) and its soluble form, sLRP-1, along with receptor for advanced glycation end products (RAGE) have also been shown to carry out transportation function of A $\beta$  across BBB (Figure 3). RAGE helps in transportation of A $\beta$  from blood into the brain while LRP-1 & sLRP-1 helps in clearance (enzymatic degradation, as well as transportation) of A $\beta$  from brain into the blood<sup>43</sup>. It has been reported in AD models and patients that the brain endothelial expression of RAGE is increased whereas the LRP expression at the BBB is reduced. This condition is unfavorable for clearance of A $\beta$  from the brain. This in turns leads to the accumulation and oligomerization of neurotoxic A $\beta$  in brain ultimately leading to neuronal death resulting in decline in cognitive ability. Besides this, it has been reported that during aging and in AD several alterations occurs in the cellular elements of the neurovascular unit and in the CP epithelia like: focal necrosis of neurovascular unit in cerebral endothelium, accumulation of extracellular matrix components in the vascular basement membrane, decrease of endothelial mitochondrial density, increase in pinocytotic vesicles, loosening of tight junctions in BBB, changes in the astrocytic endfeet and stiffening of the vessel in BBB. All these situations somehow might increase the influx of toxic A $\beta$  in brain.

### Life Style, Stress and Hormones

Aging is an inevitable journey for all and include many obstacles and different paths. The way we live our lives can have enormous impact on whether we grow old gracefully, or succumb along. It appears that keeping active, both physically and mentally, and maintaining a healthy diet, can help avoid both heart and brain disease. Stress has profound effects on the structure and function of the brain at the cellular and subcellular levels. Most of the stress-induced structural changes involve the neuronal dendrites and synaptic spines. The actin and



microtubules of dendrites and synaptic spines have been shown to change shapes in response to stress hormone<sup>44-46</sup>. Stress mediates a variety of effects on neuronal excitability, neurochemistry, and structural plasticity of the hippocampus, where  $A\beta$  is formed by APP in its membrane and then in-turn might affect cognitive ability of a AD patient.

It has been found that the stress hormone cortisol can play a role in the development of AD<sup>47</sup>. Increased peripheral and central nervous system cortisol level have also been reported in AD patients as compared to the healthy controls, which is clinically known as hypercortisolemia. Cortisol is a steroid hormone that is produced by the adrenal gland in response to stress. In case of short-term stress experience, cortisol level rapidly increases in the blood stream, and its presence is helpful in improving short-term memory formation and adapting the body's physiology to deal with the situation effectively. However the long-term stressful condition leads to the prolonged elevated levels of cortisol, which can have serious long-term effects. Various groups have reported the effect of increased cortisol in pathology of AD. It has been suggested that increased cortisol level may possible induce development of AD faster and earlier. The elevated glucocorticoid levels (mainly because of chronic stress) are correlated with increased  $A\beta$  deposition, enhanced  $A\beta$ -mediated neurotoxicity, and accelerated cognitive decline<sup>48</sup>.

It has been suggested that chronic stress-induced activation of the sympathetic nervous system leads to inflammation and there plays important role in the pathophysiology of cardiovascular disease besides many other disease<sup>49</sup>. Stress reduction, combined with a healthy lifestyle and diet will help people age successfully and avoid AD & other diseases. The structural modification in microtubules of neuronal cells causes the tau tangles formation in the brain. The alteration in protein expression in neurons in hippocampal region of brain might be one of the causes of genesis of  $A\beta$  in brain.

It is also known that women are more prone to AD. Almost two-third of Americans with AD are women<sup>2</sup>. Many facts and explanations support for more AD women patients. On average, women have longer life spans than men, and are thereby more likely to reach an age of high risk of AD. There are well-established difference in the hormonal expression between men and women, some of which may be associated with an increased risk of cognitive decline or dementia. Furthermore, women and men exhibit different forms of behavioral changes associated with the disease, possibly suggesting that the disease affects male and female brains in different ways<sup>50</sup>. This concept is supported by recent evidence from imaging studies suggesting that the disease causes structural changes in the brain that differ between men and women<sup>51</sup>. Besides all the above explanations the most prominent and accepted role is of the hormone

estrogen it seems that this hormone is needed by female brain to function normally and properly. It has been reported that many women suffer from hormonal imbalances well before middle age, as evidenced by the rise in premenstrual syndrome, polycystic ovarian syndrome (PCOS), and infertility. The risk of neurodegeneration, memory loss, dementia symptoms, and the development of AD might rise in the premenopause (the transitional stage between menstruation and menopause) condition of hormonally imbalance women. The lifetime chronic stress might also produce irregular cycles and fluctuation level of estrogen, which may ultimately leads to the AD<sup>52</sup>.

### **APOE4 Gene and Protein**

Early-onset Alzheimer's disease occurs in people age 30 to 60. It is rare, representing around 2% and have no known cause, but most cases are inherited, a type known as familial Alzheimer's disease (FAD). FAD is caused by any one of a number of different single-gene mutations on chromosomes 21, 14, and 1. These mutations cause abnormal proteins to be formed. Mutations on chromosome 21 cause the formation of abnormal APP, where as a mutation on chromosome 14 causes abnormal presenilin 1 to be made, and a mutation on chromosome 1 leads to abnormal presenilin 2. The progeny almost surely will develop FAD if anyone of the parents has AD.

Most cases of Alzheimer's are the late-onset form & developed after age 60. The causes of late-onset Alzheimer's are not yet completely understood, but they likely include a combination of genetic, environmental, and lifestyle factors that influence a person's risk for developing the disease. The ~98% cases are of LOAD (Late-onset AD) beside 2% cases of FAD (Familial AD) support the hypothesis that origin of AD might be from blood. Aging is the major risk factor for LOAD. Current studies suggested that the less physical exercise and stress factor is also a cause of this. The apolipoprotein (Apo) E4 allele on chromosome 19 is also a strong risk factor for LOAD<sup>53</sup>. The APOE gene provides the blueprint for a protein that carries cholesterol in the bloodstream. The  $\epsilon 4$  form, however, increases the risk of developing Alzheimer's disease and of developing it at a younger age. Those who inherit two  $\epsilon 4$  genes have an even higher risk. Recently it is reported in clinical trials that the low level of ApoE in plasma may have a role with genesis of Alzheimer's disease and apoE levels can be considered as biomarker for AD<sup>54</sup>. It was already suggested that long-term stress in the presence of apoE 4 allele leads to memory decline<sup>55</sup>. Possible mechanisms underlying our finding are unclear, and human studies of CSF with various Alzheimer disease-related outcomes are conflicting. It was reported that low levels of apoE both in the brain and in plasma may be early preclinical markers, because  $A\beta$  markers become abnormal up to 20 years before clinical symptoms,<sup>56</sup> and because brain apoE expression mediates clearance of  $A\beta$  peptides<sup>57</sup>. These all findings build the case of apoE involvement as an





### Platelets & CAA

The occurrence of the APP along with the secretases in other non-neuronal tissues attracted the attention of investigators. It was found that the amyloid beta proteins are also found in blood and AD was then associated with cerebrovascular disorder<sup>41</sup>. Formation & accumulation of A $\beta$  plaques in walls of fine blood vessels in brain leads to cerebral amyloid angiopathy, (CAA). In CAA, extracellular deposits, intra-neuronal lesion and neurofibrillary tangles have been noticed in blood vessels in brain parenchyma<sup>41,77,78</sup> and intraneuronal lesions - neurofibrillar tangles<sup>79</sup>. The A $\beta$  in blood is contributed by different non-neuronal organs and cells. Unlike A $\beta$ 42 deposited in senile plaques, the circulating A $\beta$  form contributing to perivascular amyloid plaques seen in CAA is primarily composed of A $\beta$ 40, which accounts for 90% of total A $\beta$  in blood<sup>80-82</sup>. A significant amount of A $\beta$ 40 is contributed by platelets. Platelets have all the enzymes to process APP including the APP protein itself & produces A $\beta$ . The major isoforms of APP are hydrolyzed by secretase enzymes to produce secreted sAPP $\alpha$  & A $\beta$ 40. Both sAPP $\alpha$  and A $\beta$ 40 can be stored in platelet alpha granules and released upon platelet activation by supporting proteins thrombin, von willibrand factor and collagen. Once A $\beta$ 40 released from activated platelets, it in turn activate more platelets. It is hypothesized that initially, in very few location aggregation of A $\beta$ 40 occurs specially in very fine blood vessels. At these locations this ultimately leads to inflammation and it is possible that due to these inflammation the BBB become leaky. Leaky BBB might be responsible for the initiation either stroke or CAA. In clinical setting, nearly, 98% AD patients develop CAA, and 75% of these patients are rated as severe CAA<sup>83</sup>. The vascular deposition of A $\beta$  is much more frequent and tends to be much more severe in patients with AD<sup>84-87</sup> than in age-matched controls. Alpha granules of platelets store biological mediators and release them at the time of activation of platelets. These mediators include chemokines such as connective tissue-activating peptide III (CTAP-III), platelet factor 4 (PF4), factor called regulated upon activation normal T-cell expressed and presumably secreted (RANTES), and macrophage inflammatory protein (MIP)-1 $\alpha$ , interleukins (IL-1 $\beta$ , IL-7, and IL-8), prostaglandins, and CD40 ligand (CD40L). Chemokine's, Interleukins might play an important role in the early pathogenesis of AD and stroke by activation of platelets and platelets activation factors. It has also been shown that the inflammatory stimulator and amyloidogenic LPS increases APP levels not only in neuronal but in non-neuronal cells as well. Decreased insulin in type 2 diabetes may also participate in APP dysregulation and A $\beta$  accumulation.

It is hypothesized that the leakiness of blood-brain-barrier must slowly increase with growing age causing major changes in brain of individuals over time. Hence A $\beta$  from platelets (or non-neuronal sources) might also be contributing to the accumulation of A $\beta$  in the brain

indirectly because of leaky BBB vasculature<sup>88-90</sup>. This also opens up the possibility that marker on platelets and other hematopoietic cells can also be potentially screened as biomarker for AD.

### Cardiovascular Diseases

Research suggests that patients with vascular disease predisposes to Alzheimer's disease<sup>91</sup>. Aggregated cardiovascular risk indices incorporating hypertension, diabetes, hypercholesterolemia and smoking increase risk are also risk factors for dementia and exposure to these risk factors increase the risk incrementally when exposure is measured in midlife<sup>92</sup> or a few years before onset of dementia<sup>93</sup>. Despite occasional negative findings from large prospective studies<sup>94,95</sup> the accumulated evidence for a causal role for cardiovascular risk factors and cardiovascular disease in the etiology of dementia and Alzheimer's disease is very strong. This has led to speculation that atherosclerosis and Alzheimer's disease are linked disease processes<sup>96</sup>, with common pathophysiological and etiologic underpinnings (APOE e4 polymorphism, hypercholesterolemia, hypertension, hyperhomocysteinemia, diabetes, metabolic syndrome, smoking, systemic inflammation, increased fat intake and obesity). The co-relation of heart disease and brain seems logical as both organ has a very elaborate and intricate network of fine capillaries. This sounds interesting, as we have already discussed the facts that the genesis of AD/Heart disease must happen much earlier than the actual appearance of clinical symptoms. A role of compromised blood brain barrier & vasculature of heart has already been discussed. So if the intricate network of blood vessels is affected in brain, similar conditions exist in cardiac vascular network. It is possible that the factors affected BBB might also, in same way affect the cardiac fine capillaries. Hence it is quite possible that risk factor affected brain might also affect heart or vice-versa.

It has been proved that the food considered healthy for cardiac health is also healthy for the brain. The omega-3 rich diet has proven to be good for the health of heart and it also affects the brain directly. It has been reported that the high intake of fat and saturated fat increased the risk of dementia and AD, whereas fish consumption, a source of omega-3 polyunsaturated fatty acids, was inversely related to the incidence of dementia, particularly AD<sup>97,98</sup>. The capillaries in brain and heart are so fine that any plaque, inflammation or leakiness will create a significant problem. If these incidences occur in only few places in such a vast network, it would be hard to detect. Detection happen only when clinical symptoms shows up. Detection in case of heart disease can be investigated by a combination of blood test, ECG & over the year much focus on cardiovascular disease by the medico's and researchers has resulted into better and early diagnosis. But there are no similar diagnostic techniques used in case of AD where, probably the genesis is almost similar. Hence a new approach of correlating cardiac theories with that of brain, in case of



AD, needs to be thought about. Any technique that could possibly map the leakiness of BBB or detect changes in fine vesicles in either head or brain could be helpful in early diagnosis.

### **Ethnic Link**

Existing evidence for AD and related dementias suggests that there are significant differences in prevalence, incidence, treatment, and mortality of Alzheimer's disease across racial and ethnic groups. There are no data recorded on AD world over but a substantial database occurs in USA & some countries in Europe. Data from USA suggested that Alzheimer's disease prevalence rates ranges from 14% to 500% higher among African Americans than among Whites<sup>99</sup>. The most frequently cited estimates are that African Americans. It has been suggested the African Americans have a tendency of about two times more and the Hispanics are about 1.5 times more likely than Whites to have Alzheimer's disease.<sup>6,2</sup>

An analysis of 2006 medicare claims data found that older African Americans and Hispanics were more likely than Whites to have a diagnosis of Alzheimer's disease. Rates were 14% for Hispanics, 13% for African Americans, 10% for Whites, 9% for Native Americans, and 8% for Asians<sup>6</sup>. In these report's the authors cautioned that prevalence rates was based on diagnosis codes & might reflect varying levels of under diagnosis across populations. World over, on the mortality based studies, the data suggests that mortality and other health outcomes among people with Alzheimer's disease vary by race and ethnicity. Despite a lower prevalence and incidence rate, Whites have a higher overall mortality rate from Alzheimer's disease in USA<sup>100,101</sup>. Rates were higher among Whites than African Americans in New England, the Mid-Atlantic, and in the East and West South Central census regions. In the East and West North Central, South Atlantic, Mountain, and Pacific regions, rates were higher among Blacks than Whites. African Americans and Latinos with Alzheimer's disease had a lower adjusted risk of mortality than their White counterparts. Asians and American Indians had similar mortality risk as Whites<sup>101</sup>. Possible reasons for racial and ethnic disparities include factors related to measurement of Alzheimer's disease, genetics, cardiovascular and cerebrovascular disease, socioeconomic factors, cultural differences, and racial and ethnic discrimination<sup>102</sup>. However clear picture is hard to emerge from the present date as it is only for a small sub of country. The ethnic diversity may be linked to the probability of occurrence of AD with its genesis linked to the genes or proteins in people with lower rate of AD. However in the absence of baseline data world over, no inference on the subject can be drawn.

### **Current Molecular Drug Targets**

Various drug targets have been summarized in Table-1. In this section the secretase enzyme family target area discussed.

### **BACE 1 as a Therapeutic Target**

Several research groups in 1999-2000 found a novel aspartic protease, BACE1, that exhibited all the previously determined characteristics of beta-secretase. Earlier after the discovery of beta secretase, its maximal activity had been observed in neuronal tissue and neuronal cell lines<sup>103</sup>. Afterwards some activity was detected in the majority of body tissues other than neuronal<sup>103</sup>. Interestingly astrocytes exhibited less beta secretase activity in than neuronal tissues.<sup>104</sup>

The levels of BACE1 mRNA are highest in brain and pancreas and are significantly lower in most other tissues. The high level of pancreatic mRNA expression was initially confusing, produced low level of BACE1 activity in tissue<sup>105</sup>. mRNA expression level were found high only in neurons but little found in resting glial cells of brain. The recent research had found the presence of BACE 1 in other non-neuronal tissues like liver, plasma, platelets and so on. The prediction and finding suggested that A $\beta$  formed in these non-neuronal tissues will cross the blood-brain barrier and accumulate in neuronal tissues of the brain and leads to AD. Platelet APP may represent the major source of A $\beta$  detected in whole blood<sup>106</sup>.

BACE1 is one of the main target protease among the researches for the treatment against AD. Since the discovery of BACE1, numerous attempts have been made to develop small-molecule BACE1 inhibitors. First-generation BACE1 inhibitors were peptide-based mimetics of the APP  $\beta$ -cleavage site, which had the APP  $\beta$ -site scissile amide bond replaced with a non-hydrolyzable transition state analog, such as statine<sup>105</sup>. More recently, nonpeptidic compounds with high affinities for BACE1 have been generated<sup>106,107</sup>. Efficacious BACE1 drugs would also need to efficiently cross the blood-brain barrier. Lot more drug companies announced the trail of drugs for BACE1 and BACE2 but their drugs failed in the phase 3 of trials.

### **$\alpha$ -secretase**

Various isoforms of  $\alpha$ -secretases have been reported like; ADAM10, ADAM 17, ADAM 9, ADAM 19 etc., a member of disintegrin and metalloprotease family. Evidence suggests that the identified  $\alpha$ -secretases demonstrate a high degree of redundancy, and which  $\alpha$ -secretases are responsible for APP cleavage in neurons and other brain cells is still being debated<sup>108,109</sup>. It was found that any stimulator of  $\alpha$ -secretase is hard to develop. Because  $\alpha$ -secretase activity is regulated by the involvement of known pathways of Protein kinase C, mitogen-activated protein kinases, tyrosine kinases and calcium-mediated, and developing compounds that stimulate  $\alpha$ -secretase via these pathways is clearly possible<sup>110</sup> but the problem is that those target pathways are involved in several other base line signaling in almost all cells. Many treatment and drugs based on stimulation of  $\alpha$ -secretase has also being targeted indirectly and tested in clinical trials. In fact, evidence that indirect activation of  $\alpha$ -secretase is





successfully achieved by  $\alpha$ -7-nicotinic acetylcholine receptor agonists, a 5-hydroxytryptamine receptor 4 agonist, and a  $\gamma$ -aminobutyric acid. A receptor modulator has been used to support the clinical development of these agents. In many incidences data had shown that these compounds increase  $\alpha$ -secretase-mediated cleavage of APP and reduce  $A\beta$  levels *in vivo*<sup>111</sup> and but it has been reported that attenuation of AD symptoms by these drugs has not been demonstrated in patients.

### **$\gamma$ -secretase**

The full inhibition of  $\gamma$ -secretase appeared to be a sound approach. However, it was found that  $\gamma$ -secretase plays a broader biological role and cleaves multiple proteins to yield physiologically essential products. Unlike  $\beta$ -secretase,  $\gamma$ -secretase has proved to be a highly tractable target for AD drug treatment, at least with respect to the development of orally bioavailable, brain-penetrant  $\gamma$ -secretase inhibitors (GSIs)<sup>112</sup>. Recently in 2014 Eli Lilly & Co. and Bristol-Myers Squibb had previously developed inhibitors—a drug that block  $\gamma$ -secretase. However, in 2010 and 2012, both companies halted clinical trials of the drugs because they actually increased the rate of cognitive decline.

### **Possible Drug Targets**

The processes involved in genesis & development of AD are not only due to tau proteins and secretase enzymes present in the brain but other factors, which may be the true factors for the genesis of AD. These factors may be the receptors and leaky BBB with the proteins involved for the transportation of  $A\beta$ , the current irregular life style, which may increasing the stress and deregulation of hormones, the level of APOE4 gene and protein, the occurrence of type-II diabetes and alteration in the function of pancreatic cells in patients with AD, the vascular factors like platelets activation during normal conditions, the high rate of cardiovascular disease, ethnic link with AD and so on.

### **Recent Non-Invasive Method**

Recent research of University of Queensland research institute suggested a non-pharmacological approach for removing  $A\beta$  and restoring memory function in a mouse model of AD. Non-invasive method has therapeutic results without the need of additional therapeutic agents such as anti- $A\beta$  antibody. This recent research reported the clearance of  $A\beta$  plaques through microglial cells by using iterations process of scanning ultrasound. The ultrasound process resulted in restoration of memory function to the same level of normal healthy mice<sup>113</sup>.

### **DISCUSSION/CONCLUSION**

After the formation of  $A\beta$  fibrils and tangles due to tau proteins, AD slowly develops into a wide systemic disorder. It is reported that the formation of “curly fibers” and “tangles” is not unique to the central nervous system<sup>114</sup>. Atherosclerosis, hypertension, diabetes mellitus, and lipids are major cardiovascular disease risk

factors have been shown to be associated with AD<sup>72</sup>. Strong epidemiologic evidence suggests an association between AD and type 2 diabetes<sup>71,115-117</sup>. The percentage of type 2 diabetes among AD patients is significantly higher than among age-matched non-AD controls<sup>118,71</sup>. Conversely, patients with type 2 diabetes have twice the risk of controls to develop AD<sup>72-76</sup>. Although the blood-brain barrier (BBB) is able to protect the CNS from immune activation, it becomes more permeable during inflammation, which renders the brain vulnerable to infections.

In regards to chronic neurodegenerative disorders such as Alzheimer's disease, the brains of patients with Alzheimer's disease have been reported to show BBB leakage that was associated with the APOE4 allele, suggesting that BBB dysfunction may contribute to the onset and/or progression of AD. Several novel studies are now focusing upon the critical role that the BBB and vascular disease in development of chronic neurodegenerative disorders. A report by the Ning examine the role of cerebral microemboli during  $\beta$ -amyloid deposition in an amyloid precursor protein/presenilin 1 (APP/PS1) double transgenic mouse model of Alzheimer's disease<sup>119</sup>.

The authors demonstrated that internal carotid artery injection of microemboli can result in increased  $\beta$ -amyloid deposition that occurs during elevated matrix metalloproteinase-9 expression, suggesting that BBB injury may be a factor leading to the increased  $\beta$ -amyloid accumulation in the brain. Interestingly, Provias and Jeynes also illustrated that in the brains of Alzheimer's patients, capillary vascular endothelial growth factor is significantly reduced in the superior temporal, hippocampus and brainstem regions of the brain. Given the significant role of vascular endothelial growth factor for endothelial cell viability and BBB maintenance, their work provides further support for the premise that vascular disease in conjunction with BBB injury may be a contributory factor for the development or progression of Alzheimer's disease.

In conjunction with vascular injury, inflammatory disease also may be a significant factor that severely impacts neurodegenerative cell injury<sup>120</sup>.

All these evidences and reports suggest the transportation through blood may be a possible cause of AD other than its beginning and occurrence in brain. It is also suggested that the stress of daily life due to current lifestyle might also be a factor for early onset of AD. Hence, a fresh perspective is needed & a new thought process is required to provide much needed impetus to future research for treating AD.



**Table 1:** Different drugs produced and different outcomes of drugs for Alzheimer's disease till date.

(Source for the data in table 1: J. A. Mikulca PharmD, Journal of Clinical Pharmacy and Therapeutics, 2014, 39, 25–37)

Status of secretase inhibitors and/or modulators.					
Drug name (Co. designation) trial name sponsor [Clinicaltrials.gov registry number]	Phase	Mechanism of Action	Primary and secondary outcomes.	Status	Outcomes
EPOCH@Merck [NCT01739348] EudraCT Number 2008-005260-14	II/III	$\beta$ -secretase inhibitor	Primary: ADAS-Cog, ADCS-ADL Secondary: safety	Started November 2012, currently enrolling	n.a.
Pirenzepine (ACI-91)@AC Immune SA	II	$\beta$ -secretase modulator	Primary: safety and tolerability Secondary: cognitive effects	Started March 2010, discontinued January 2013	General, but statistically not significant, decrease in measures of cognitive performance and clinical function
Semagacestat (LY450139) IDENTITY@Eli Lilly [NCT00594568]	III	$\gamma$ -secretase inhibitor	Primary: ADAS-Cog, ADCS-ADL Secondary: safety, QoL	tarted March 2008, discontinued August 2010	Treated patients worsened to a statistically significant greater degree than placebo patients
Semagacestat (LY450139) IDENTITY-2 Eli Lilly [NCT00762411]	III	$\gamma$ -secretase inhibitor	Primary: ADAS-Cog, ADCS-ADL Secondary: safety, QoL	Started March 2008, discontinued August 2010	Treated patients worsened to a statistically significant greater degree than placebo patients
Avagacestat (BMS-708163) n.a. Bristol-Myers Squibb [NCT00810147]	II	$\gamma$ -secretase inhibitor	Primary: safety and tolerability Secondary: PD, PK	Started February 2009, completed June 2010	Lower doses well tolerated with mild GI and dermatologic side effects; dose-dependent cases of non-melanoma skin cancer and worsening cognitive function
CHF-5074Chiesi Pharmaceuticals [NCT01303744]	II	$\gamma$ -secretase modulator	Primary: safety and tolerability Secondary: PK	Started March 2011, completed US: April 2012, Italy: ongoing	n.a.
NIC5-15/n.a./ Humanetics Pharmaceuticals [NCT00470418]	II, a, b	Selective $\gamma$ -secretase inhibitor (direct and indirect)	Primary: safety and tolerability Secondary: ADAS-Cog, MMSE, ADCS-ADL	Started January 2007, completed March 2010	n.a.
CTS-21166 CoMentis [NCT00621010]	I	$\beta$ -secretase inhibitor	Primary: safety and tolerability Secondary: PK	Started June 2007, completed February 2008	Dose-dependent reductions in plasma Ab levels >60% as measured by AUC over 24 h or as maximum reduction relative to predose levels; to date, no further studies planned
E2609 Eisai [NCT01294540]	I	$\beta$ -secretase inhibitor	Primary: safety and tolerability Secondary: PK	Started December 2010, completed October 2011	Dose-dependent reduction in plasma Ab levels; results led to a further trial (phase 1, currently recruiting [NCT01600859])
E2609 Eisai [NCT01511783]	I	$\beta$ -secretase inhibitor	Primary: safety and tolerability Secondary: PK	Started December 2011, completed July 2012	Reduction in plasma Ab levels, dose-dependent reduction in Ab CSF levels; results led to a further trial (phase 1, currently recruiting [NCT01600859])
LY2811376 Eli Lilly [NCT00838084]	I	$\beta$ -secretase inhibitor	Primary: safety and tolerability Secondary: PK	Started December 2008, completed June 2009	Dose-dependent reduction in plasma Ab levels; however, further studies not



					completed due to retinal toxicity in rats
LY2886721 Eli Lilly [NCT01561430]	I/II	$\beta$ -secretase inhibitor	Primary: safety and tolerability Secondary: ADAS-Cog, CDR-SB, MMSE	Started March 2012, currently enrolling	n.a.
<b>Other Approaches status</b>					
Bapineuzumab ELN115727-301 Pfizer/Janssen [NCT00574132]	III	Humanized monoclonal antibody binds to and clears Ab	Primary: cognitive and function over 18 month Secondary: cognitive and global function, imaging and biochemical biomarkers of disease status over 18 month	Started December 2007, completed June 2012	2 large-scale trials failed primary outcome results as of August 2012
Gammagard Liquid [(immune globulin intravenous (IGIV 10%)]/ Gammaglobulin Alzheimer's Partnership (GAP) Baxter Healthcare Cooperation Alzheimer's Disease Cooperative Study (ADCS) [NCT00818662]	III	Binds to Ab including oligomers and fibres to promote clearance	Primary: cognition and global function using ADAS-Cog, ADCS, ADL for 18 mo Secondary: cognition and global function over 9 month	Started December 2008, completed December 2012	Not reported
Solanezumab (LY2062430) EXPEDITION EXPEDITION 2 Eli Lilly	III	Humanized anti-Ab immunoglobulin G-1 (IgG1) monoclonal antibody	Primary: changes in baseline to endpoint in ADAS-Cog11, ADCS-ADL Secondary: change from baseline to endpoint in CDR-SB, NPI, vMRI, MMSE, RUD-Lite, EQ-5D Proxy, QoL-AD, plasma Ab levels	Started 2009, completed April 2012	Failed primary outcome results
TRX-0237 TauRx therapeutics [NCT01689246]	III	Tau aggregation inhibitor	Primary: change from baseline in ADCS-CGIC, change in ADAS-cog11, no. of participants who tolerate oral TRx0237 Secondary: change from baseline in MMSE	Started December 2012, currently enrolling	N/A
ACC-001 with QS-21 [NCT00960531] Pfizer/Janssen	II	Peptide vaccine attached to a carrier protein, using the surface-active saponin adjuvant QS-21, intended to help induce an antibody response against AD	Primary: incidence and severity of treatment emergent AEs and clinically important changes in safety assessments Secondary: change from baseline levels of anti-Ab IgG, anti-Ab IgM and IgG subclass antibody levels at selected time points	Started July 2009, completion September 2014	N/A
Affitope AD02 Affiris AG [NCT01117818]	II	Vaccine, active component is a synthetic peptide functionally mimicking the unmodified N- terminus of Ab	Primary: cognitive (ADAS-cog modified) and functional (ADCS-ADL modified) Secondary: cognitive (computerized test battery), global (CDR-sb), behavioural (NPI), biomarker (volumetric MRI)	Started April 2010 Estimated completion date March 2014	Ongoing
CAD106 Novartis [NCT01097096]	II	Vaccine to Promote clearance of Ab by the induction of antibodies against Ab	Primary: safety and tolerability (physical & neurological examinations, ECG, vital signs, standard and special laboratory evaluations, MRIs, AEs/serious AEs monitoring) Secondary: Ab-specific and Qb carrier-specific antibody response in serum and CSF or T-cell response using peripheral blood	Started March 2010 Completed December 2012	Not reported



			mononuclear cells (PBMcs); changes in levels of disease-related markers (e.g. Ab1-40 and Ab1-42 in plasma; Ab1-40, Ab1-42, total-tau, phospho-tau in CSF)		
Crenezumab (MABT5102A) Genentech [NCT01723826]	II	Humanized IgG4 monoclonal antibody to promote Ab clearance	Primary: frequency and severity of AEs, vital signs/physical findings, neurological findings, laboratory test results, incidence of human anti-therapeutic antibody (ATA) formation, incidence of ARIA-E and amyloid-related imaging abnormalities-haemorrhage	Started December 2012	Not reported
Gantenerumab (R04909832) DOMINANTLY INHERITED ALZHEIMER NETWORK TRIAL Washington University School of Medicine; Eli Lilly, Roche, Alzheimer's Association [NCT01760005]	II	Humanized monoclonal antibody to promote Ab clearance	Primary: amount of fibrillar amyloid deposition measured by [ <sup>11</sup> C] PIB PET scans Secondary: change in CSF Ab levels, change in CSF biomarkers tau and ptau181 values compared between subjects on active drug, rate of brain atrophy in treatment groups vs. pooled placebo group, change in 2- [ <sup>18</sup> F] fluoro-2- deoxy-D-glucose (FDG) PET metabolism in specific regions of interest, measured for 2 years	Started December 2012 Estimated completion December 2016	N/A
BMS-241027 Bristol-Myers Squibb [NCT01492374]	I	Microtubule-stabilizing agent that also has effects on tau protein in animal models of AD	Primary: safety (based on frequency of serious AEs, discontinuation due to AEs and dose reduction), CSF levels of tau N-terminal domain fragments Secondary: effects on CSF levels of tau fragment, cognitive performance, connectivity MRI, Cmax, plasma concentration at 24 h, Tmax, AUC, safety, effects on CSF levels of neurofilaments	Started February 2012 Currently enrolling.	
V950 Merck [NCT00464334]	I	Vaccine Ab amino-terminal peptides conjugated to ISCO- MATRIX™	No. with ≥ 1 AE, no. who discontinued study drug due to AE, geometric mean titre (GMT) of Ab 1-40 antibodies at 7 month, mean fold change from baseline in GMT of Ab 1-40 antibodies at 7 month	Started March 2007 completed in January 2012	



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