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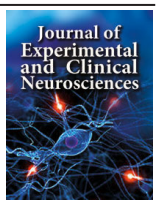

Invited Editorial

Combating Detrimental Aging-related Uprisings: Impacts of Immunotherapy and Vaccination

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Abstract

Of central nervous system (CNS) disorders, Alzheimer's disease (AD) continues to grow among elderlies causing a big challenge for the health systems worldwide. While no conventional therapies can cure the AD-mediated CNS disorders such as dementia, passive and active vaccination of this disease seem to be a promising preventive treatment modality. Technically, the immunotherapy of AD is based on either active immunization through injection of amyloid beta42 (Abeta42) as antigen, or passive immunization by injection of anti-Abeta42 monoclonal antibodies (mAb). So far, some peptides (e.g. CAD106, ACC001 and Affitope) and mAbs (e.g. solanezumab, gantenerumab, and crenezumab) have even been taken into phase 2/3 clinical trials. Despite promising clinical outcomes from these trials, it seems that we are yet to engineer effective immunomedicines to fight the aging-related CNS disorders (e.g. AD-mediated dementia). Here, in this mini-review, we discuss the hallmarks of AD and highlight the essentiality of this imperative challenge.

Keywords: *Alzheimer's disease, Aging, CNS disorders, Immunotherapy, Vaccination*

Introduction

Conceptually, immunization/vaccination is one of the most effective approaches in combating most infectious and/or contagious diseases as well as any other diseases that are manifestoed by over-expression of undesired biomarkers. Recently, numerous studies have been conducted worldwide to fight life-threatening diseases such as malignancies and age-related CNS disorders using personalized immunomedicienes. This dominion still needs some advancement towards production and application of effective personalized immunotherapies targeting array of biomarkers involved in development of the CNS-mediated disabling diseases such as AD-mediated dementia. Of note, AD is a disabling CNS disorder that creates a lot of pressure on the health care systems in modern societies where life expectancy is high and so is the aging-mediated diseases. As the most common basis of dementia among elderlies, AD is known as one of the major contributors to disability and dependency worldwide. The immunization/vaccination of AD needs a better understanding of molecular pathology of the disease which may promote development of new personalized vaccination and cell therapy modalities.

Although the initiation, development and progression of AD are not fully comprehended, the basis of AD pathogenesis is deemed to be due to the accumulation of abnormally folded amyloid-beta protein (Aβ) and/or hyperphosphorylated tau protein (ptau) in the brain. It is worthy of noting that the AD is most common cause of dementia that contributes in approximately 60-70% of the cases, and is considered as one of the major priorities of public health worldwide. Furthermore it results in devastating disabilities contributing to morbidity among elderlies. AD requires substantial health-care interventions. Unfortunately, the number of patients with dementia is estimated to be roughly doubled every 20 years (i.e., 66 million in 2030 and 115 million in 2050) [1]. Further, the cell-based vaccination and immunotherapy modalities granted great hopes. It is deemed that these initiatives will change the face of therapy towards personalized medicines for most of the human diseases such as age-related disorders. Nowadays, the reprograming of human cells is no longer a fiction and in reality we are able to isolate the desired cells from any individuals, remodel the molecular features and inject them back. Despite being in its infancy era, this approach seems to be the only rational treatment of some diseases even though we need to devise much more effective modalities based on a holistic panel of intricate phenomena specific for each individual.

Since in human aging a complex network of biomolecules are involved, preventive vaccination and immunotherapy may bestow a cost-effective and officious means to overcome these hurdles.

To tackle this issue, various preventive, alleviative and treatment modalities have been conducted, including: medications for memory loss, behavioral and sleep changes. All these therapies are aimed to target several biomolecules that are believed to be involved in development of AD. This review provides some insights on the the hallmarks of AD and also discusses inevitable challenges in fighting the AD-mediated dementia.

Hallmarks of Alzheimer's disease

Among several biomarkers involved in initiation/development of AD, amyloid beta (Abeta) and tau proteins play central roles and are briefly discussed in relation with inflammation.

Amyloid beta

In the mid-1980s, after discovery of Abeta, formation of plaques through aggregation of Abeta peptides was considered as an important hallmark of Alzheimer's brain abnormality [2]. Abeta is the chief component of plaques that, together with neurofibrillary tangles (NFTs) because of tau protein aggregation, causes the age-related neurodegenerative disorders in particular AD. Taken all investigations into consideration, the parent amyloid precursor protein (APP), which is a transmembrane protein consisting of 695–770 amino acids, is deemed to be sequentially cleaved by a group of enzymes complexes – known as α-, β- and γ-secretases. APP enzymatic cleavage non-amyloidogenic (i.e., by α and γ-secretases at a position 83 amino acids from the carboxy terminus) and amyloidogenic

(i.e., by β- and γ-secretases at a position 99 amino acids from the carboxy terminus) pathways [3]. While the non -amyloidogenic pathway precludes the formation of Abeta, the amyloidogenic pathway leads to the generation of intact Abeta peptides as approximately 90% of 40 residues in length (Aβ40) and about 10% of 42 residues in length (Aβ42). The latter peptide seems to be more hydrophobic entity and hence more prone to fibril formation than Aβ40, resulting in formation of cerebral plaques. Several enzymes are involved in an intricate activities of α-, β- and γ-secretases. The α- secretase functionality is based on the enzymatic activity of A Disintegrin And Metalloproteinase (ADAM) family (i.e., ADAM9, ADAM10 and ADAM17) and possesses metalloprotease, integrin-binding, intracellular signaling and cell adhesion activities [4-6]. The β-secretase enzymatic activity is mediated by APP- cleaving enzyme 1 (BACE1) that is a type I integral membrane protein of the pepsin family of aspartyl proteases [7,8]. And finally, the γ-secretase was reported to be a complex of enzymes encompassing presenilin 1 or 2 (PS1, PS2), anterior pharynx defective, nicastrin and presenilin enhancer 2.

Tau protein

Tau protein is responsible for development of neurofibrillary tangles (NFTs) that occur intraneuronally. It was discovered in mid-1970s, since then a large number of researchers showed its impact in initiation and progression of AD. In the late 1980s, NFTs were shown as the aggregates of the tau proteins, abnormally hyperphosphorylated forming tangles in the brain parenchyma. Tau protein belongs to the microtubule-associate proteins (MAPs) and its encoding gene locates on the long arm of chromosome 17 (i.e., at 17q21) encompassing 16 exons. This protein can stabilize the microtubules which seems to occur as coordinated actions of kinases and phosphatases, in which the phosphorylation of tau protein modulates its interaction with microtubules impacting the axonal transportation of vesicles along the microtubules mediated by kinesin protein [9]. Conformationally, tau protein shows six different isoforms whose sizes vary from 352 to 441 amino acid residues. In AD, it has been shown that this protein fails to maintain the cytoskeleton of the axonal bodies well-organized in large part due to altered microtubules-binding capacity (perhaps because of conformational changes and/or misfoldings) [10,11]. For instance, in the neurons affected in AD, ptau has been reported to lose the binding capacity to the tubulin while failing to promote the assembly of microtubule, and hence, precluding the organization of microtubule within the affected neurons [12,13]. Such inhibitory impacts on vesicular trafficking within the neuronal cells appears to affect the exocytosis functions and mitochondrial dissemination. Pathologically, the abnormal ptau seems to trigger the in situ aggregation of ptau in AD patients. Further, proteolytic cleavage of the tau protein is of note phenomena which seems to be an alternative mechanism for the occurrence of the abnormal aggregation of tau [14]. All these aberrant events may lead to some inevitable neuronal loss within the areas of the brain that are responsible for the cognitive functions. Also it should be noted that aberrant alteration in tau protein structure (e.g., abnormal phosphorylation, acetylation, glycation, nitration, truncation) are deemed to play a pivotal role in development pf AD; readers are directed to see [10,15-17].

Inflammation

Inflammation has also been reported to be a central player in Alzheimer's brain abnormality. In fact, development of Abeta-based plaques and ptau-mediated tangles seems to sustain chronic activation of primed microglia, activating some important inflammatory functions and hence producing inflammatory bio-agents such as cytokines and chemokines by the affected cells. Such aberrant phenomena are deemed to impair the microglia and also impact the neighboring cells such as astrocytes, oligodendrocytes and neurons, which can result in neurodegeneration and neuronal loss. In neurodegenerative diseases, the core contribution of the innate immune system occurs via resident microglia and perivascular macrophages, while other blood-derived myeloid cells (e.g., dendritic cells and monocytes) impose little influence upon the initiation/progression neurodegeneration phenomena [18]. Having highlighted all these, the inflammatory impacts in AD are yet to be fully understood; readers are directed to see [18-20].

Insulin and insulin receptor impacts

In AD – apart from less than 5% of all cases due to mutations of three genes that result in the permanent development of disease – all other cases are sporadic in origin related to aging. AD is often associated with some anomalous engagements with the neuronal insulin signaling pathway, in which Abeta seems to bind to the insulin receptor competitively in the early-onset familial AD while this receptor can be desensitized in the late-onset sporadic AD by noradrenaline and/or cortisol [21]. In addition to the glucose metabolism, there exist compelling evidence that insulin and insulin receptor modulate the metabolism of APP, the intracellular formation and the release of APPs and Abeta into the extracellular space, and also phosphorylation of tau protein [21-24]. Taken all, we must fully comprehend the role of insulin in the brain and address issues such as how brain cells get their energy needs. For example, the glucose insufficiency in neuronal system could be moderately compensated through the use of endogenous brain substrates (i.e., amino acids and fatty acids). Accordingly, in the early-onset familial AD, glutamate is metabolized to retain the ATP level at a normal state, while the use of glutamate as an energy source may inadvertently result in production of neurotoxic ammonia in the brain and thus interfere with normal function of mitochondria [21]. All these findings suggest that we need to implement new strategies to stave off AD, for which personalized immunotherapy seems to bestow much more effective means.

Blood-brain barrier (BBB) impacts in AD development

Principally, in healthy condition, the BBB control the traverse of endogenous and exogenous compounds into the brain and hence maintains the homeostasis of the central dogma. In the presence of astrocytes and pericytes, the brain microvasculature endothelial cells form a very tight barrier setting with great integrity by creating tight junctions consisting of proteins such as zonula occluden; readers are directed to see [25-30]. However, in AD, calcium influx is induced by Aβ resulting in perturbed intracellular Ca2+ homeostasis and hence loss of BBB integrity [31,32].

Immunization of Alzheimer's disease

Generally, it is of essential demands to control the dreadful AD ideally by active or passive immunization. Further, it should be highlighted that the stem cells isolated and transplanted into the intact brain of rodents were shown to be able to pursue their right trajectory in the host parenchyma resulting in (a) differentiation into functional neural lineages, (b) targeted relocation towards the damaged regions of the brain, and (c) proliferation and maturation towards functional neural cells. The intravenously administered neural precursor cells are also able to migrate into the damaged areas of brain and induce functional recovery [33-36]. These findings have raised several clinical trials to attain the proof-of-concept in human subjects (e.g., ClinicalTrials.gov Identifier: NCT01547689). While the stem cell therapy of AD has just been begun to combat the dementia, a large number of studies have been conducted to examine the potentials and benefits of the immunotherapy and vaccination modalities (Table 1).

To the best of our knowledge, the immunotherapy of AD has been capitalized on (a) active immunization against amyloid β42 (Abeta42), in which patients receive injections of the antigen itself, or (b) passive immunization in which patients receive injections of monoclonal antibodies (mAb) against Abeta42. Of the examined active or passive immunizations approaches, some of the peptide-based vaccines used for active immunizations

were CAD106, ACC001 and Affitope that have been undergone phase 2/3 clinical trials. In addition, some monoclonal antibodies (mAbs) such as solanezumab, gantenerumab, and crenezumab have been investigated in phase 2 and 3 clinical trials [1], which have resulted in some limited clinical benefits (Table 2).

Altogether, it appears that there is an immediate need for an initiative advocating the advancement of investigation on the immunization of AD. While the currently used medicines for AD simply alleviate the symptoms of AD-mediated dementia, recently studies on active/passive immunotherapy appear to be treatment strategies.

Further, targeted therapy of AD has been shown to successfully reduce the accumulation of Abeta, resulting in profound prevention of downstream

Table 1. Selected clinical trials conducted for immunotherapy and vaccination of Alzheimer's disease.

Study	Identifier	Biological	Phase	Status
Study to Evaluate Safety, Tolerability and Immunogenicity of Vaccine (UB 311) in Subjects With Alzheimer's Disease	NCT00965588	UB 311	1	Completed (Aug 2011)
18−months Safety Follow−up Study of AADvac1, an Active Tau Vaccine for Alzheimer's Disease (FUNDAMANT)	NCT02031198	AADvac1	1	Currently recruiting
Study Evaluating Single Ascending Doses of AAB−001 Vaccine SAD Japanese Patients With Alzheimers Disease	NCT00397891	Bapineuzumab	1	Completed (Aug 2014)
Study Evaluating Safety, Tolerability, And Immunogenicity Of ACC−001 In Subjects With Mild To Moderate Alzheimer's Disease	NCT00479557	ACC−001 and QS−21	2	Completed (Feb 2013)
Phase Ib Follow−up Study to Evaluate Long−term Safety and Tolerability of Immunization With AFFITOPE AD01 Applied During AFFiRiS 001	NCT00711139	AFFITOPE AD01 and AFFiRiS 001	1	Completed (Dec 2009)
To Investigate the Safety and Tolerability of Repeated Subcutaneous Injections of CAD106 in Alzheimer's Patients	NCT01023685	CAD106	2	Completed (June 2013)
AAB−001 in Patients With Mild to Moderate Alzheimer's Disease	NCT00112073	Bapineuzumab	2	Completed (March 2012)
Safety Study of Passive Immunization for Patients With Mild to Moderate Alzheimer's Disease	NCT00174525	AAB−001	2	Unknown
Effect of LY2062430 on the Progression of Alzheimer's Disease (EXPEDITION2)	NCT00904683	LY2062430	3	Completed (Dec 2012)
Progress of Mild Alzheimer's Disease in Participants on Solanezumab Versus Placebo (EXPEDITION 3)	NCT01900665	Solanezumab	3	Currently recruiting

Note: Data were obtained from https://clinicaltrials.gov.

Table 2. Biologics used for passive or active immunization of AD.

Biologic	Description	Immunity	Manufacturer	Clinical impacts
CAD106	Multiple copies of Aβ1-6 peptide derived from the N-terminal B cell epitope of Aβ, coupled to a Qβ virus-like particle	Active	Novartis Pharmaceuticals, Corp. (Basel, Switzerland)	
ACC001	A conjugate of multiple short Aβ fragments linked to a carrier made of inactivated diphtheria toxin	Active	Janssen Pharmaceuticals, Inc. (Titusville, NJ, USA)	Discontinued after phase 2 trials
Affitope (AD01)	Short peptides, mimicking parts of the native beta amyloid (Aβ) sequence as the antigenic component	Active	AFFiRiS, (Vienna, Austria)	Successful completed clinical phase 1 studies, Under phase 2 studies
Affitope (AD02)	A synthetic peptide of six amino acids that mimics the N-terminus of Aβ, that lacks the most common T cell epitope (amino acids 15-42 of Aβ) but including the B cell epitope (amino acids 11-15 of Aβ) , it may allow for the production of anti-Aβ antibodies while minimizing a pro-inflammatory TH1 response	Active	AFFiRiS, (Vienna, Austria)	Increased production of anti-Aβ Ab, decreased TH1 response (successful phase 1), Under phase 2 studies
AN-1792	Synthetic full-length Aβ peptide with QS-21 adjuvant	Active	Janssen Pharmaceuticals, Inc. (Titusville, NJ, USA); Pfizer Inc. (New York City, NY, USA)	Discontinued after phase 2 trials
Solanezumab	A humanized monoclonal whole antibody	Passive	Eli Lilly (Indianapolis, IN, USA)	Failed in phase 3 trials, limited benefits
Gantenerumab	An investigational fully human mAb against Aβ	Passive	Roche (Basel, Switzerland)	Failed in phase 3 trials, limited benefits
Crenezumab	A humanized whole mAb targeting human 1-40 and 1-42 Aβ	Passive	AC Immune (Lausanne, Switzerland), Genentech, Inc. (San Francisco, CA, USA)	Failed in phase 3 trials, limited benefits
Bapineuzumab	A humanized monoclonal whole antibody against Aβ	Passive	Pfizer Inc. (New York City, NY, USA); Johnson & Johnson (New Brunswick, NJ, USA)	Failed in phase 3 trials, limited benefits

pathology of the disease. Both immunotherapies using antibodies against AN-1792 peptide and ptau protein indicate their clinical benefits even though such impacts seem to be more effective in early stages of amyloid accumulation. Despite promising clinical impacts of the active vaccination with Abeta peptide, clinical trials using this strategy were halted in part because of the development of meningoencephalitis in number of patients treated with such modality. Although the active vaccine therapy and passive immunization using humanized anti-Abeta mAbs are judged to be effective on the basis of clinical and pathological analyses in some patients, their overall clinical outcomes are still under question. This review highlights some benefits and challenges of the active vaccination and passive immunotherapy against AD.

Passive immunization through administration of few humanized anti-Abeta mAbs has been taken into clinical trials even though these immunotherapies have still some issues to be solved. Of note, DNA vaccines have been developed to combat AD. This approach seems to be a simple strategy that can be easily modified and administered with no need for any types of adjuvants. Several research groups have capitalized

on DNA vaccines based on Abeta targeting, upon which significant reduction has been shown for the induced Abeta in AD model mice without side effects. It should be noted that AD as a neurodegenerative disorder is under intensive investigation towards development of effective immunotherapy modalities.

Further, there exist a significant correlation between pathology of AD and accumulation of extracellular Abeta or intracellular ptau fibrils which can be therapeutic targets for intrabody immunotherapy; readers are directed to see [37]. Paganetti and co-workers reported on targeting of the intercellular pathway of Abeta production through an intrabody, sFvβ1, which was directed to the EFRH epitope, a tetrapeptide next to the β-secretase cleavage site of β-amyloid precursor protein. While sFvβ1 binds to newly synthesized APPs in endoplasmic reticulum and escorts it during secretory transport to membrane, it protects APP from the pathologic β-secretase-mediated cleavage, a process producing toxic Abeta [38]. In addition, a large panel of anti-tau intrabodies were produced to target the microtubule-associated protein tau, involved in AD. However, in vivo studies and therapeutic efficacy of all above intrabodies remains to be determined.

As a long-standing goal of AD research, our today's challenges and even tomorrow's confrontations seem to be the personalized prevention of diseases through reprograming of the immune system. The strategy can be applied against life-affecting and age-related diseases such as malignancies [39] and inflammatory disorders. However, for the development of such modalities, we will need to advance our knowledge upon the mechanism(s) of these diseases in a holistic manner. To be precise, targeting a single marker of fatal diseases no longer can be the case, but rather a cascade of key molecular markers need to be targeted by the endogenous defense machineries of patient, even far before the onset of the disease through implementation of the cell-mediated immunotherapy and vaccination as novel personalized holistic therapy modality.

References

1. Jindal H1, Bhatt B, Sk S, Singh M.J.: Alzheimer disease immunotherapeutics: then and now. Hum Vaccin Immunother 2014, 10(9): 2741-3.
2. Glenner G.G, Wong C.W: Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 1984, 120(3): 885-90.
3. LaFerla F.M, Green K.N, Oddo S: Intracellular amyloid-beta in Alzheimer's disease. Nat Rev Neurosci 2007, 8(7): 499-509.
4. Allinson TM, Parkin ET, Turner AJ, Hooper NM: ADAMs family members as amyloid precursor protein alpha-secretases. J Neurosci Res 2003, 74(3): 342-52.
5. Duffy M.J, et al: The ADAMs family of proteins: from basic studies to potential clinical applications. Thromb Haemost 2003, 89(4): 622-31.
6. Yang P, Baker K.A, Hagg T: The ADAMs family: coordinators of nervous system development, plasticity and repair. Prog Neurobiol 2006, 79(2): 73-94.
7. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, et al: Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 1999, 286(5440): 735-41.
8. Cole S.L, Vassar R: The role of amyloid precursor protein processing by BACE1, the beta-secretase, in Alzheimer disease pathophysiology. J Biol Chem 2008, 283(44): 29621-5.
9. Kolarova M, García-Sierra F, Bartos A, Ricny J, Ripova D: Structure and pathology of tau protein in Alzheimer disease. Int J Alzheimers Dis 2012. 2012: 731526.
10. Cardenas A.M, Ardiles AO, Barraza N, Baéz-Matus X, Caviedes P: Role of tau protein in neuronal damage inAlzheimer's disease and Down syndrome. Arch Med Res 2012:43(8): 645-54.
11. Morozova O.A, March ZM, Robinson AS, Colby DW: Conformational features of tau fibrils from Alzheimer's disease brain are faithfully propagated by unmodified recombinant protein. Biochemistry 2013, 52(40): 6960-7.
12. Avila J, Lim F, Moreno F, Belmonte C, Cuello AC: Tau function and dysfunction in neurons: its role in neurodegenerative disorders. Mol Neurobiol 2002, 25(3): 213-31.
13. Mi K, Johnson G.V: The role of tau phosphorylation in thepathogenesis of Alzheimer's disease. Curr Alzheimer Res 2006,3(5):449-63.
14. Wischik C.M, Novak M, Thøgersen HC, Edwards PC, Runswick MJ, Jakes R: Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. Proc Natl Acad Sci U S A 1988, 85(12): 4506-10.
15. Haroutunian V, Davies P, Vianna C, Buxbaum JD, Purohit DP: Tau protein abnormalities associated with the progression of alzheimer disease type dementia. Neurobiol Aging 2007, 28(1): 1-7.
16. Yan S.D, Chen X, Schmidt AM, Brett J, Godman G, Zou YS, et al: Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. Proc Natl Acad Sci U S A 1994, 91(16): 7787-91.
17. Papasozomenos S.C, Su Y. Altered phosphorylation of tau protein in heat-shocked rats and patients with Alzheimer disease. Proc Natl Acad Sci U S A 1991, 88(10): 4543-7.
18. Heppner F.L, Ransohoff R.M, Becher B: Immune attack: therole of inflammation in Alzheimer disease. Nat Rev Neurosci 2015,16(6): 358-72.
19. Golde T.E: Inflammation takes on Alzheimer disease. Nat Med 2002, 8(9): 936-8.
20. Weninger S.C, Yankner B.A: Inflammation and Alzheimer disease: the good, the bad, and the ugly. Nat Med 2001, 7(5): 527-8.
21. Hoyer S: Glucose metabolism and insulin receptor signal transduction in Alzheimer disease. Eur J Pharmacol 2004, 490(1-3): 115-25.
22. Mentis M.J, Weinstein EA, Horwitz B, McIntosh AR, Pietrini P, Alexander GE, et al: Abnormal brain glucose metabolism in the delusional misidentification syndromes: a positron emission tomography study in Alzheimer disease. Biol Psychiatry 1995, 38(7): 438-49.
23. Guze B.H, Baxter LR Jr, Schwartz JM, Szuba MP, Mazziotta JC, Phelps ME, et al: Changes in glucose metabolism in dementia of the Alzheimer type compared with depression: a preliminary report. Psychiatry Res 1991, 40(3): 195-202.
24. Hoyer S, Oesterreich K. Wagner O: Glucose metabolism as the site of the primary abnormality in early-onset dementia of Alzheimer type? J Neurol 1988, 235(3): 143-8.
25. Omidi Y, Campbell L, Barar J, Connell D, Akhtar S, Gumbleton M: Evaluation of the immortalised mouse brain capillary endothelial cell line, b.End3, as an in vitro blood-brain barrier model for drug uptake and transport studies. Brain Res 2003, 990(1-2): 95-112.
26. Smith M, Omidi Y, Gumbleton M: Primary porcine brain microvascular endothelial cells: biochemical and functional characterisation as a model for drug transport and targeting. J Drug Target 2007, 15(4):253-68.
27. Omidi Y, Barar J, Ahmadian S, Heidari HR, Gumbleton M: Characterization and astrocytic modulation of system L transporters in brain microvasculature endothelial cells. Cell Biochem Funct 2008, 26(3): 381-91.
28. Barar J, Gumbleton M, Asadi M, Omidi Y: Barrier functionality and transport machineries of human ECV304 cells. Med Sci Monit 2010, 16(1): BR52-60.
29. Nakhlband A, Omidi Y: Barrier functionality of porcine and bovine brain capillary endothelial cells. Bioimpacts 2011, 1(3): 153-9.
30. Omidi Y, Barar J: Impacts of blood-brain barrier in drug delivery and targeting of brain tumors. Bioimpacts 2012, 2(1): 5-22.
31. Kook S.Y, Seok Hong H, Moon M, Mook-Jung I: Disruption of

blood-brain barrier in Alzheimer disease pathogenesis. Tissue Barriers 2013, 1(2): e23993.

32. Kalaria R.N: The blood-brain barrier and cerebral microcirculation in Alzheimer disease. Cerebrovasc Brain Metab Rev 1992, 4(3): 226-60.
33. Abdel-Salam O.M: Stem cell therapy for Alzheimer's disease. CNS Neurol Disord Drug Targets 2011, 10(4): 459-85.
34. Li X, Y, Bao X.J, Wang R.Z: Potential of neural stem cell- based therapies for Alzheimer's disease. J Neurosci Res 2015.
35. Li M, Guo K, Ikehara S: Stem cell treatment for Alzheimer's disease. Int J Mol Sci 2014, 15(10): 19226-38.
36. Young J.E, Goldstein L.S. Alzheimer's disease in a dish: promises and challenges of human stem cell models. Hum Mol Genet 2012, 21(R1): R82-9.
37. Citron M: Alzheimer's disease: strategies for disease modification. Nat Rev Drug Discov 2010, 9(5): 387-98.
38. Paganetti P, Calanca V, Galli C, Stefani M, Molinari M: beta-site specific intrabodies to decrease and prevent generation of Alzheimer's Abeta peptide. J Cell Biol 2005,168(6): 863-8.
39. Barar J, Omidi Y: Personalized cell-mediated immunotherapy and vaccination: combating detrimental uprisings of malignancies. Bioimpacts 2015, 5(2): 65-69.