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Review

Delaying Dementia: Targeted Brain Delivery Using Lipid Cubic Phases

Joseph S. D'Arrigo [‡, *]

Cavitation-Control Technology Inc., Farmington, CT 06032, USA; E-Mail: cavcon@ntplx.net

‡ Current Affiliation: Cav-Con Inc., Bellevue, WA 98007, USA

* **Correspondence:** Joseph S. D'Arrigo; E-Mail: cavcon@ntplx.net

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Abstract

Microvascular endothelial dysfunction precedes, often by decades, the cognitive decline associated with Alzheimer's disease. Hence, preservation of a healthy cerebrovascular endothelium can be an important therapeutic target. By incorporating appropriate drug(s) into biomimetic (lipid cubic phase) nanocarriers, one obtains a multitasking combination therapeutic which targets certain cell-surface scavenger receptors, mainly class B type I (i.e., SR-BI), and crosses the blood-brain barrier. Documented similarities in lipid composition – among high-density lipoproteins (HDL) and the biomimetic (nanoemulsion) nanocarrier particles – can partially simulate or mimic the known heterogeneity (i.e., subpopulations or subspecies) of HDL particles. Such colloidal-nanocarrier targeting allows for various Alzheimer's-related cell types to be simultaneously searched in a holistic integrative approach, in vivo, for localized drug treatment. Using various biobased lipids and their mixtures to form self-assembled non-lamellar nanostructures, it has continually been reported possible to successfully obtain stable colloidal dispersions of (liquid-crystalline) lipid cubic phases with well-defined particle size and morphology. In particular, monoglyceride-based lyotropic liquid-crystalline phases are relatively unique owing to their rich polymorphism in water and potential application as drug nanocarriers. This (colloidal-

nanocarrier) in vivo targeting advantage may be particularly important when delivering pleiotropic natural substances (e.g., an isoflavone) or for repurposing an FDA-approved drug.

Keywords
Alzheimer's disease; blood-brain barrier; cognitive decline; dementia; drug targeting; lipid cubic phases; nanoemulsion; scavenger receptors; SR-BI

1. Background

Vascular brain lesions are very common in people over 70 years old, and recent reviews [1, 2] provide much evidence that a large proportion of dementia cases may be attributable to cerebrovascular disease [3, 4]. Accordingly, vascular cognitive impairment and dementia (VCID) is the second leading cause of dementia behind Alzheimer's disease, and is a frequent co-morbidity in the Alzheimer's patient [5, 6]. Furthermore, growing data from brain imaging studies and various animal models suggest that cerebrovascular dysfunction may well preceed cognitive impairment and the onset of neurodegenerative changes in Alzheimer's disease [2, 4].

2. Endothelial Dysfunction, and Targeted Nanotherapy for Early Dementia

Small-vessel disease is commonly found in patients with neurodegenerative disease, such as Alzheimer's patients. The vascular changes associated with small-vessel disease include a blood-brain barrier (BBB) breakdown with leakage of blood-borne molecules [4]. It is no surprise, therefore, that multiple epidemiological studies have shown a marked overlap among risk factors for small-vessel cerebrovascular disease and late-onset Alzheimer's disease.

It has been reported repeatedly that *endothelial* modulation and repair is feasible by pharmacological targeting [1, 2, 7-13] via SR-BI receptors [13]. As the detailed review by Mahringer et al. [14] points out, the BBB is equipped with several endocytic receptors at the luminal surface (i.e., the capillary endothelial membrane), including SR-BI. Recently, Fung et al. [15] specifically found that SR-BI mediates the uptake and transcytosis of HDL across brain microvascular endothelial cells (i.e., across the BBB). Since SR-BI has already been identified as a major receptor for HDL (with their major apolipoprotein *(apo)A-I) as well as for* the recently reviewed [1,2] "lipid-coated microbubble/nanoparticle-derived" *(LCM/ND) nanoemulsion* (see below), this multitasking lipid nanoemulsion can arguably serve as a targeted, apoA-I-based, (SR-BI mediated) therapeutic agent for common (late-onset) dementias [16-18].

This targeted-delivery-approach, using the proposed LCM/ND lipid nanoemulsion for treating the more common (late-onset) dementias, receives added impetus from continual findings of cerebrovascular pathology [1, 19-29] and an apparent *endothelium* dysfunction [2, 17, 18, 25, 30-36] in both Alzheimer's disease and its major risk factors [1, 2, 29-41]. By incorporating drug molecules into the LCM/ND lipid nanoemulsion type (yielding particle sizes mostly < 0.1 µm in diameter – see Figure 1), known to be a successful drug carrier [42, 43], one is likely to obtain a multitasking combination therapeutic capable of targeting cell-surface SR-BI. This (intravenous) combination therapeutic would make it possible for various cell types, all potentially implicated in

Alzheimer's disease [1, 2], to be simultaneously sought out and better reached for localized drug treatment of brain tissue in vivo [42, 43].

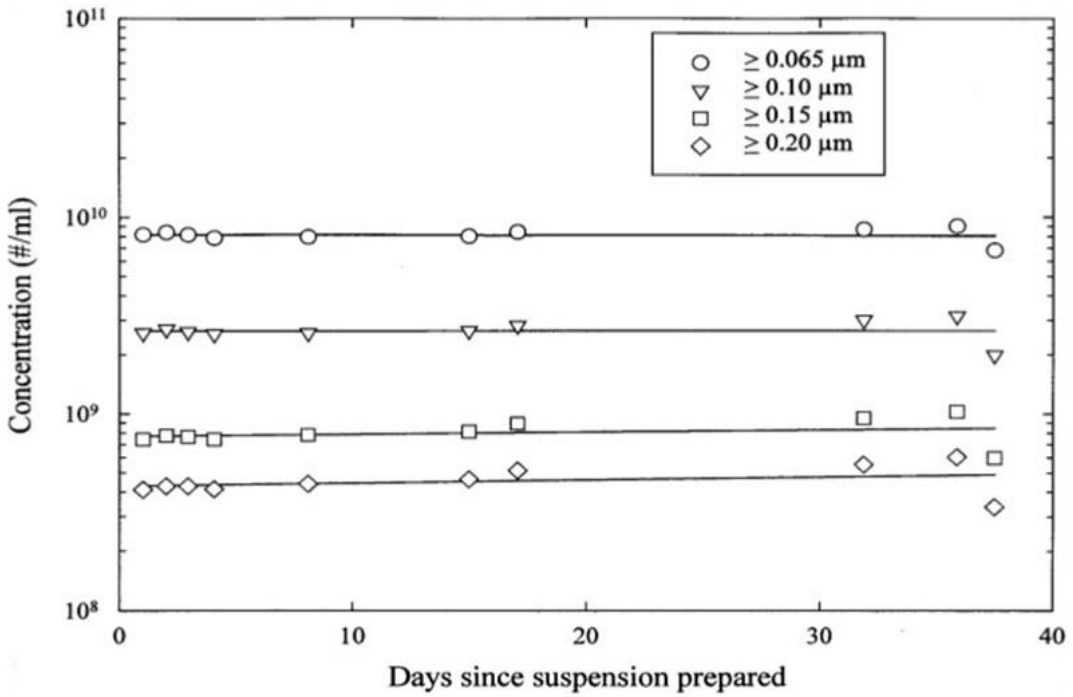

Figure 1 LCM/ND nanoemulsion stability over time. (Adapted from [2])

3. LCM/ND Nanoemulsion Type, and Targeting via Lipid Cubic Phases

Importantly, monoglyceride is the largest single-lipid fraction (by wt. %) of the powdered solid lipid surfactants used to produce the (Filmix®) LCM/ND nanoemulsions [42]. As a group, monoglycerides exhibit different phase behaviors when they are exposed to water [44, 45]. In particular, the self-assembly of varied and useful *dispersed cubic* phases (among other liquid-crystalline phases) depends heavily on the acyl chain length of the glycerides (primarily monoglycerides) placed in contact with water [42]. The (lyotropic or solvent-induced) cubic liquid-crystalline phases may be classified into two distinct classes: *bi*continuous cubic phases *and* micellar or *dis*continuous (e.g., type *Fd3m*) cubic phases [46-50].

The *dispersed Fd3m* cubic phase can represent a lipid/water system which is particularly relevant to the earlier-described (Filmix®) LCM/ND lipid nanoemulsion formulation(s) on account of the fact that the patent claims describing the precise lipid composition of such nanoemulsion formulations (see especially Claim #1 in [51, 52]) specifically include cholesterol and three categories of (saturated) glycerides, that is, tri-, di-, and monoglycerides [51, 52]. In view of the advantageous attributes of monoglycerides (recounted above), and since (saturated) monoglyceride represents the largest single-lipid fraction of the LCM/ND lipid nanoemulsion type, the monoglyceride content probably plays a dominant role in supporting the evident long-term stability of the liquid-crystalline lipid nanoparticles in such nanoemulsions [42].

In this particular targeted-delivery approach, the self-assembled “lipid particle” structure itself (upon intravenous injection of the LCM/ND nanoemulsion) is apparently successfully utilized as

the "active" targeting ligand – which is directed via (adsorption of) plasma lipoproteins (including notably apoA-I) toward the appropriate receptors on the target-cell surface [42].

4. Amyloid-β Ion Channel Hypothesis of Alzheimer's Disease

As explained in many reviews [53, 54] by different investigators, it has been recognized for over two decades that disturbance of the intracellular calcium homeostasis is central to the pathophysiology of several neurodegenerative disorders. As concerns Alzheimer's disease, it is believed by many researchers that enhanced calcium load may be brought about by extracellular accumulation of amyloid-β (Aβ) in the brain. Such studies have laid the foundation for the popular idea that amyloid-β peptides (39-42 amino acid molecules) are, in part, toxic to brain tissue because they form aberrant ion channels in cellular membranes and thereby disrupt Ca^{2+} homeostasis in brain tissue and increase intracellular Ca^{2+} [53, 54].

Historical support for the above amyloid-β ion channel hypothesis, or so-called "calcium hypothesis", has also been observed at the clinical level [55]. Namely, there is little correlation between the amounts of fibrillar (insoluble) deposit at autopsy and the clinical severity of Alzheimer's disease. In contrast, a good correlation exists between early cognitive impairment and levels of soluble forms of Aβ in the brain [56]. (Aggregation of Aβ proceeds from formation of soluble (low molecular weight) spherical oligomers toward eventually assuming a final and stable conformation as insoluble fibrils from which amyloid-β plaques are constituted.) Hence, neurotoxicity is associated with soluble aggregates (i.e., oligomers) of Aβ rather than with the plaques themselves [56].

As summarized by Di Scala et al. [55], the structure of amyloid pores has been extensively studied by ultrastructural methods. In particular, one group of investigators recently applied strategies (widely used to examine the structure of membrane proteins) to study the two major Aβ variants, namely, Aβ (1-40) and Aβ (1-42). Under the optimized detergent micelle conditions: 1) Aβ (1-40) aggregated into amyloid fibrils; 2) contrariwise, Aβ (1-42) assembled into oligomers that inserted into lipid bilayers as well-defined pores [57]. (These amyloid pores adopted characteristics of a β-barrel arrangement.) Because Aβ (1-42), relative to Aβ (1-40), has a more prominent role in Alzheimer's disease, the higher propensity of Aβ (1-42) to form β-barrel pore-forming oligomers is an indication of their importance in Alzheimer's disease [57]. Very recently, a different research group reported very similar findings [58]. As background for their study, these latter authors point out that: – elevated Aβ (1-42) plasma levels have been correlated with the progression of late-onset forms of Alzheimer's disease; Aβ (1-42) is significantly more neurotoxic than Aβ (1-40) both in vivo and in neuronal cell culture; and memory impairment is believed to be driven by Aβ (1-42) disruption of long-term (hippocampal) potentiation. In accordance with these considerations, the authors' own detailed experimental data [58] indicated that Aβ (1-42) assemblies in oligomeric preparations form ion channels (in membranes excised from cells of neuronal origin). In contrast, Aβ (1-40) oligomers, fibrils, and monomers did not form channels. Moreover, ion channel conductance results suggested that Aβ (1-42) oligomers, but not monomers and fibrils, formed pore structures. The authors concluded that their findings demonstrate that only Aβ (1-42) contains unique structural features that facilitate membrane insertion and channel formation, now aligning ion channel formation with the neurotoxic effect of Aβ (1-42) compared to Aβ (1-40) in Alzheimer's disease [58].

5. Promise of Bexarotene (or Analogs) to Inhibit Cognitive Decline in Humans

The preceding discussion of amyloid pore formation, in the cellular membranes of brain tissue, leads to another important consideration – the role of cholesterol. Namely, cholesterol is required for the assembly of amyloid pores formed by Aβ (1-42) [55]. Therefore, an amphipathic drug (such as bexarotene) which competes with cholesterol for binding to Aβ (1-42) would be capable of preventing oligomeric channel formation (at least in vitro). Such a strategy has already been contemplated earlier for the treatment of Alzheimer's and other neurodegenerative diseases that involve cholesterol-dependent toxic oligomers [59].

At least two recently published reports (both in 2017) on bexarotene indicate a continuing interest in the ability of this FDA-approved (anticancer) drug to: 1) bind free Aβ peptide, as well as 2) bexarotene's previously reported positive effects in Alzheimer's-disease mouse models [60, 61]. Because it is the first drug that can both inhibit the binding of cholesterol to Aβ (1-42) and prevent calcium-permeable amyloid pore formation in the plasma membrane of brain cells, bexarotene might be considered as the prototype of a new class of anti-Alzheimer compounds [62]. (Note that because bexarotene shares structural analogy with cholesterol, and the above-described LCM/ND nanoemulsion contains substantial concentrations of cholesterol esters and cholesterol (see above), incorporation of the bexarotene molecule into the LCM/ND nanocarrier is expected to represent an uncomplicated, straightforward formulation procedure commercially.) Moreover, Casali et al. [63] have very recently reported that treatment of an Alzheimer's-disease mouse model with (this FDA-approved anticancer drug) bexarotene resulted in enhanced cogniton in the APP/PS1 mice which resembled earlier findings. Strikingly, the authors observed sustained cognitive improvements in the mice even when bexarotene treatment was discontinued for 2 weeks. Also, they observed a sustained reduction in microgliosis and plaque burden, following drug withdrawal, exclusively in the hippocampus. Casali et al. concluded that bexarotene selectively modifies aspects of neuroinflammation in a region-specific manner to reverse cognitive deficits in Alzheimer's-disease (APP/PS1) mice [63].

Additional molecular aspects, concerning the membrane-related mechanisms for the known neuroprotective effect, of bexarotene action on brain tissue continue to be suggested and/or described in the recent literature [64-66]. In the most recently published study, Kamp et al. [66] show by NMR and CD spectroscopy that bexarotene directly interacts with the transmembrane domain of amyloid precursor protein (APP) – as similarly suspected for Aβ peptides (see below). The longer, neurotoxic, Aβ (1-42) peptide is highly aggregation prone and represents the major Aβ species deposited in the brain [66-69]. Cholesterol promotes Aβ (1-42) aggregation by enhancing its primary nucleation rate by up to 20-fold [69]. Earlier work by Di Scala et al. [59] provided evidence that it is possible to prevent the generation of neurotoxic oligomers by targeting the cholesterol-binding domain of Aβ peptides [59]. Therefore, blocking of the initial (soluble-)Aβ triggering event, by employing a drug such as bexarotene (or an analog), can be seen as a crucial goal in treating dementia early. Note that such blocking of amyloid-β-induced neurotoxic pore formation can be expected to avoid exacerbation of blood-brain barrier breakdown, already occurring at lower levels in aged humans with cognitive decline [70], and thereby prevent reaching higher levels of BBB breakdown associated with cognitive impairment (and/or eventually dementia) in late-onset Alzheimer's disease [70-72]. The known neuroprotective effect of beraxotene action on brain tissue has also recently stimulated expanded research into the use of

bexarotene derivatives (i.e., analogs) [73-75], which demonstrated the successful attenuation of Alzheimer's-related pathologies and cognitive impairments in an Alzheimer's-disease mouse model [75].

6. Targeted Delivery of Genistein to Delay Dementia

Genistein is pleiotropic molecule that engages several different mechanisms/pathways to enhance brain health – including reduction of oxidative stress, promotion of growth factor signaling, and immune suppression. These physiological actions occur in endothelial, glial, and neuronal cells to provide a coordinated beneficial action to ischemic/hypoxic challenge [76].

Using a model of Alzheimer's disease in vitro (by exposing primary hippocampal neurons of newborn rats either to oxygen/glucose deprivation or to amyloid-β peptide), researchers [77-79] have recently observed the effects of genistein (a type of soybean isoflavone) on such exposed rodent cells – with regard to neuron viability and electrophysiological properties of voltage-gated sodium channels and potassium channels in these hippocampal neurons. These researchers determined that genistein partially reversed the decrease in hippocampal neuron viability, after the above exposure, as well as the induced alterations in voltage-activated sodium and potassium currents. These authors concluded that their studies suggest that genistein may exert some neuroprotective effects via modulation of electrophysiological properties of voltage-activated sodium channels and potassium channels [77-79].

Moreover, in separate experiments it has recently been reported by other investigators [80] that genistein shows strong ability to prevent the conformational transition of amyloid-β monomers to β-sheet structures. The resultant finding from these experiments – employing atomic force microscopy and circular dichroism – was that genistein reduced the final amyloid fibrillization (from amyloid-β monomer aggregation) by 40-63%. Furthermore, comparative molecular dynamics simulations revealed that genistein prefers to bind the β-sheet groove of amyloid-β oligomers, which then interfers with their self-aggregation [80].

Lastly, in earlier work [81] (using a cell-based screening model for CLA-1 [the human ortholog of SR-BI] up-regulators), Yang et al. have shown genistein able to up-regulate CLA-1 transcriptional activity in the cell-based reporter assay [81]. Accordingly, in view of the above-described central role of the CLA-1/(SR-BI) receptor in targeted-delivery behavior of the LCM/ND nanocarrier (see 2nd section), any such up-regulation of this receptor could be of considerable interest/importance when contemplating the possible success of a (human) clinical trial evaluating the effect of targeted-delivery of genistein, on early dementia, using the proposed LCM/ND nanoemulsion delivery vehicle.

7. Other Drug Candidates for Targeted Brain Delivery in order to Delay Dementia

Three other low-molecular-weight, and sufficiently lipophilic, candidates for incorporation into the LCM/ND lipid nanoemulsion are docosahexaenoic acid (DHA), astaxanthin, and poly-hydroxybutyric acid (PHB).

DHA is the subject of several, very recent reports on this molecule's protective effects against Alzheimer's disease [82-85]. Specifically, when different fish polyunsaturated fatty acids (PUFAs) were administered to a senescence-accelerated mouse model of (sporadic) Alzheimer's disease, only DHA was found able to reverse the cognitive deficits (i.e., memory deficits) in these mice [83].

In the most recent report on the mechanistic effects of DHA against Alzheimer's pathophysiology, Eto et al. argue that DHA may affect the fibrillization of Aβ peptides [82, 84, 85]. (Note that formation of amyloid fibrils consists of two stages, i.e., the initial nucleation phase and the following elongation phase.) More specifically, these authors found that DHA accelerated the formation of Aβ fibrils with a unique short and curved morphology in its nucleation phase. These short and curved Aβ fibrils formed in the presence of DHA did not facilitate the elongation phase of Aβ fibril formation, indicating a possible mechanism of how DHA acts protectively against the pathophysiology of Alzheimer's disease [82].

As concerns the second drug candidate mentioned at this section's start, astaxanthin has very recently been reported to exert protective effects similar to bexarotene in animal models of Alzheimer's disease [86]. (Note that astaxanthin, a carotenoid, is a lipid-soluble plant pigment. It is an FDA-approved food coloring, and is generally recognized as safe (GRAS) by the FDA.) Using porcine brain capillary endothelial cells (pBCEC), Fanaee-Danesh et al. found that astaxanthin (as did bexarotene) enhanced Aβ clearance to the apical/plasma compartment of this in vitro BBB model [86]. In parallel, astaxanthin reduced levels of Aβ oligomers in murine BCEC, and Aβ species in brain soluble and insoluble fractions, from an Alzheimer's-disease mouse model. From their various findings, these authors conclude that astaxanthin (as well as bexarotene) exerts beneficial effects at the BBB by balancing cholesterol homeostasis and enhancing clearance of Aβ from blood-brain barrier endothelial cells [86].

The third drug candidate is β-hydroxybutyric acid (or 3-hydroxybutyric acid), which can be readily utilized to form hydrophobic poly-hydroxybutyric acid (PHB) polymers for use in nanoparticle drug-delivery systems (e.g., [87]). In particular, targeted brain delivery by incorporation of hydrophobic PHB polymers into the LCM/ND lipid nanoemulsion makes delaying dementia plausible. Past reviews by other investigators (e.g., [88]) make clear that Alzheimer's pathology involves all of the major elements of the neurovascular unit of the mature Alzheimer brain – the neurons, glia, and blood vessels. Clinically, reduced glucose utilization, decades before cognitive deterioration, reveals ongoing energy insufficiency. β-hydroxybutyrate can provide energy to the brain when glucose utilization is blocked. Early work in mouse models of Alzheimer's disease demonstrated the ability of β-hydroxybutyrate to reverse the pathological changes in the Alzheimer brain, and initial clinical trials reveal its ability to improve cognition and everyday function [88].

8. Conclusion

The above-described multitasking (drug-delivery) therapeutic could represent a promising way to treat, delay, or even prevent Alzheimer's disease in the future [1, 2, 89]. Specifically, by incorporating the appropriate drug(s) into biomimetic (lipid cubic phase) nanocarriers, one obtains a multitasking combination therapeutic which targets certain cell-surface scavenger receptors, mainly class B type I (SR-BI), and crosses the BBB. Such biomimetic-nanoemulsion targeting allows for various Alzheimer's-related cell types to be simultaneously searched out, in vivo, for localized drug treatment. This (colloidal-nanocarrier) in vivo targeting advantage may be particularly important when delivering pleiotropic natural substances (e.g., an isoflavone) or for repurposing FDA-approved food additive(s) and/or drug(s).

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Competing Interests

The author declares no conflict of interest. J.S.D. is employed at Cav-Con Inc.

References

1. D'Arrigo JS. Alzheimer's disease, brain injury, and CNS nanotherapy in humans: Sonoporation augmenting drug targeting. Med Sci. 2017; 5: 29.
2. D'Arrigo JS. Nanotherapy for Alzheimer's disease and vascular dementia: Targeting senile endothelium. Adv Colloid Interface Sci. 2018; 251: 44-54.
3. Tariq S, d'Esterre CD, Sajobi TT, Smith EE, Longman RS, Frayne R, et al. A longitudinal magnetic resonance imaging study of neurodegenerative and small vessel disease, and clinical cognitive trajectories in non-demented patients with transient ischemic attack: The PREVENT study. BMC Geriatr. 2018; 18: 163.
4. Stefanova NA, Maksimova KY, Rudnitskaya EA, Muraleva NA, Kolosova NG. Association of cerebrovascular dysfunction with the development of Alzheimer's disease-like pathology in OXYS rats. BMC Genomics. 2018; 19: 75.
5. Kalaria RN. Neuropathological diagnosis of vascular cognitive impairment and vascular dementia with implications for Alzheimer's disease. Acta Neuropathol. 2016; 131: 659-685.
6. Duncombe J, Kitamura A, Hase Y, Ihara M, Kalaria RN, Horsburgh K. Chronic cerebral hypoperfusion: A key mechanism leading to vascular cognitive impairment and dementia. Closing the translational gap between rodent models and human vascular cognitive impairment and dementia. Clin Sci. 2017; 131: 2451-2468.
7. Srimanee A, Regberg J, Hallbrink M, Vajragupta O, Langel U. Role of scavenger receptors in peptide-based delivery of plasmid DNA across a blood-brain barrier model. Int J Pharm. 2016; 500: 128-135.
8. Di Marco LY, Venneri A, Farkas E, Evans PC, Marzo A, Frangi AF. Vascular dysfunction in the pathogenesis of Alzheimer's disease - a review of endothelium-mediated mechanisms and ensuing vicious circles. Neurobiol Dis. 2015; 82: 593-606.
9. Carradori D, Gaudin A, Brambilla D, Andrieux K. Application of nanomedicine to the CNS diseases. Int Rev Neurobiol. 2016; 130: 73-113.
10. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. Neurobiol Dis. 2016; 107: 41-56.
11. Qosa H, Mohamed A, Al Rihani SB, Batarseha YS, Duong QV, Keller JN, et al. High-throughput screening for identification of blood–brain barrier integrity enhancers: A drug repurposing opportunity to rectify vascular amyloid toxicity. J Alzheimers Dis. 2016; 53: 1499-1516.
12. Koizumi K, Wang G, Park L. Endothelial dysfunction and amyloid-induced neurovascular alterations. Cell Mol Neurobiol. 2016; 36:155-165.

13. Goldwaser EL, Acharya NK, Sarkar A, Godsey G, Nagele RG. Breakdown of the cerebrovasculature and blood-brain barrier: A mechanistic link between diabetes mellitus and Alzheimer's disease. J Alzheimers Dis. 2016; 54: 445-456.
14. Mahringer A, Reichel V, Ott M, MacLean C, Reimold I, Hollnack-Pusch E, et al. Overcoming the blood brain barrier: The challenge of brain drug targeting. J Nanoneurosci. 2012; 2: 5-19.
15. Fung KY, Wang C, Nyegaard S, Heit B, Fairn GD, Lee WL. SR-BI mediated transcytosis of HDL in brain microvascular endothelial cells is independent of caveolin, clathrin, and PDZK1. Front Physiol. 2017; 8: 841.
16. Robert J, Button EB, Stukas S, Boyce GK, Gibbs E, Cowan CM, et al. High-density lipoproteins suppress Aβ-induced PBMC adhesion to human endothelial cells in bioengineered vessels and in monoculture. Mol Neurodegener. 2017; 12: 60.
17. Robert J, Stukas S, Button E, Cheng WH, Lee M, Fan J, et al. Reconstituted high-density lipoproteins acutely reduce soluble brain Aβ levels in symptomatic APP/PS1 mice. Biochim Biophys Acta 2016; 1862: 1027-1036.
18. Hottman DA, Chernick D, Cheng S, Wang Z, Li L. HDL and cognition in neurodegenerative disorders. Neurobiol Dis. 2014; 72: 22-36.
19. Weekman EM, Sudduth TL, Caverly CN, Kopper TJ, Phillips OW, Powell DK, et al. Reduced efficacy of anti-A immunotherapy in a mouse model of amyloid deposition and vascular cognitive impairment comorbidity. J Neurosci. 2016; 36: 9896-9907.
20. Nelson AR, Sweeney MD, Sagare AP, Zlokovic BV. Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. Biochim Biophys Acta. 2016; 1862: 887-900.
21. Kapasi A, Schneider JA. Vascular contributions to cognitive impairment, clinical Alzheimer's disease, and dementia in older persons. Biochim Biophys Acta. 2016; 1862: 878-886.
22. McAleese KL, Alafuzoff I, Charidimou A, De Reuck J, Grinberg LT, Hainsworth AH, et al. Post-mortem assessment in vascular dementia: Advances and aspirations. BMC Med. 2016; 14: 129.
23. Noh Y, Seo SW, Jeon S, Lee JM, Kim JS, Lee JH, et al. The role of cerebrovascular disease in amyloid deposition. J Alzheimers Dis. 2016; 54: 1015-1026.
24. Hishikawa N, Fukui Y, Sato K, Kono S, Yamashita T, Ohta T, et al. Cognitive and affective functions in Alzheimer's disease patients with metabolic syndrome. Eur J Neurol. 2016; 23: 339-345.
25. Gutierrez J, Honig L, Elkind MS, Mohr JP, Goldman J, Dwork AJ, et al. Brain arterial aging and its relationship to Alzheimer dementia. Neurology. 2016; 86: 1507-1515.
26. Nagata K, Yamazaki T, Takano D, Maeda T, Fujimaki Y, Nakase T, et al. Cerebral circulation in aging. Ageing Res Rev. 2016; 30: 49-60.
27. Calabrese V, Giordano J, Signorile A, Ontario ML, Castorina S, de Pasquale C, et al. Major pathogenic mechanisms in vascular dementia: Roles of cellular stress response and hormesis in neuroprotection. J Neurosci Res. 2016; 94: 1588-1603.
28. Toth P, Tarantini S, Csiszar A, Ungvari ZI. Functional vascular contributions to cognitive impairment and dementia: Mechanisms and consequences of cerebral autoregulatory dysfunction, endothelial impairment, and neurovascular uncoupling in aging. Am J Physiol Heart Circ Physiol. 2017; 312: H1-H20.

29. Devraj K, Poznanovic S, Spahn C, Schwall G, Harter PN, Mittelbronn M, et al. BACE-1 is expressed in the blood-brain barrier endothelium and is upregulated in a murine model of Alzheimer's disease. J Cereb Blood Flow Metab. 2016; 36: 1281-1294.
30. Chao AC, Lee TC, Juo SH, Yang DI. Hyperglycemia increases the production of amyloid-peptide leading to decreased endothelial tight junction. CNS Neurosci Ther. 2016; 22: 291-297.
31. Khalil RB, Khoury E, Koussa S. Linking multiple pathogenic pathways in Alzheimer's disease. World J Psychiatry. 2016; 6: 208-214.
32. Festoff BW, Sajja RK, van Dreden P, Cucullo L. HGMB1 and thrombin mediate the blood-brain barrier dysfunction acting as biomarkers of neuroinflammation and progression to neurodegeneration in Alzheimer's disease. J Neuroinflamm. 2016; 13: 194.
33. Gangoda SV, Butlin M, Gupta V, Chung R, Avolio A. Pulsatile stretch alters expression and processing of amyloid precursor protein in human cerebral endothelial cells. J Hypertens. 2016; 34: e24.
34. Roberts AM, Jagadapillai R, Vaishnav RA, Friedland RP, Drinovac R, Lin X, et al. Increased pulmonary arteriolar tone associated with lung oxidative stress and nitric oxide in a mouse model of Alzheimer's disease. Physiol Rep. 2016; 4: e12953.
35. Kyrtsos CR, Baras JS. Modeling the role of the glymphatic pathway and cerebral blood vessel properties in Alzheimer's disease pathogenesis. PLoS ONE. 2015; 10: e0139574.
36. Kalaria, RN, Akinyemi R, Ihara M. Stroke injury, cognitive impairment and vascular dementia. Biochim Biophys Acta. 2016; 1862: 915-925.
37. Khan A, Kalaria RN, Corbett A, Ballard C. Update on vascular dementia. J Geriatr Psychiatry Neurol. 2016; 29: 281-301.
38. Toda N, Okamura T. Cigarette smoking impairs nitric oxide-mediated cerebral blood flow increase: implications for Alzheimer's disease. J Pharmacol Sci. 2016; 131: 223-232.
39. Uiterwijk R, Huijts M, Staals J, Rouhl RP, De Leeuw PW, Kroon AA, et al. Endothelial activation is associated with cognitive performance in patients with hypertension. Am J Hypertens. 2016; 29: 464-469.
40. Wang YJ. Lessons from immunotherapy for Alzheimer's disease. Nat Rev Neurol. 2014; 10: 188-189.
41. Krstic D, Knuesel I. Deciphering the mechanism underlying late-onset Alzheimer's disease. Nat Rev Neurol. 2013; 9: 25-34.
42. D'Arrigo JS. Stable nanoemulsions: Self-assembly in nature and nanomedicine. Amsterdam: Elsevier. 2011; 25: 415.
43. Barbarese E, Ho SY, D'Arrigo JS, Simon RH. Internalization of microbubbles by tumor cells in vivo and in vitro. J Neurooncol. 1995; 26: 25-34.
44. Garg G, Saraf Sh, Saraf Sw. Cubosomes: An overview. Biol Pharm Bull. 2007; 30: 350-353.
45. Pouton CW. Properties and uses of common formulation lipids, surfactants and cosurfactants. Proceedings of the AAPS workshop-effective utilization of lipid-based systems to enhance the delivery of poorly soluble drugs: Physicochemical, biopharmaceutical and product development considerations; 5-6 March 2007; Bethesda, MD, USA. Arlington, VA: AAPS; 2007; Constantinides PP, Porter CJH, Eds.
46. Kaasgaard T, Drummond CJ. Ordered 2-D and 3-D nano-structured amphiphile self-assembly materials stable in excess solvent. Phys Chem Chem Phys. 2006; 8: 4957-4975.
47. Small DM. The behavior of biological lipids. Pure Appl Chem. 1981; 53: 2095-2103.

48. Seddon JM, Robins J, Gulik-Krzywicki T, Delacroix H. Inverse micellar phases of phospholipids and glycolipids. Phys Chem Chem Phys. 2000; 2: 4485-4493.
49. Luzzati V, Vargas R, Mariani P, Gulik A, Delacroix H. Cubic phases of lipid-containing systems: Elements of a theory and biological connotations. J Mol Biol. 1993; 229: 540-551.
50. Luzzati V, Vargas R, Gulik A, Mariani P, Seddon JM, Rivas E. Lipid polymorphism: a correction. The structure of the cubic phase of extinction symbol Fd – consists of two types of disjointed reverse micelles embedded in a three-dimensional hydrocarbon matrix. Biochemistry. 1992; 31: 279-285.
51. D'Arrigo JS. Surfactant mixtures, stable gas-in-liquid emulsions, and methods for the production of such emulsions from said mixtures. U.S. Patent. No. 4,684,479; issued 1987.
52. D'Arrigo JS. Method for the production of medical-grade lipid-coated microbubbles, paramagnetic labeling of such microbubbles and therapeutic uses of microbubbles. U.S. Patent No. 5,215,680; issued 1993.
53. Nimmrich V, Eckert A. Calcium channel blockers and dementia. Brit J Pharmacol. 2013; 169: 1203-1210.
54. Shirwany NA, Payette D, Xie J, Guo Q. The amyloid beta ion channel hypothesis of Alzheimer's disease. Neuropsychiatr Dis Treat. 2007; 3: 597-612.
55. Di Scala C, Yahi N, Boutemeur S, Flores A, Rodriguez L, Chahinian H, et al. Common molecular mechanism of amyloid pore formation by Alzheimer's β-amyloid peptide and α-synuclein. Sci Rep. 2016; 6: 28781.
56. Demuro A, Smith M, Parker I. Single-channel Ca^{2+} imaging implicates Aβ1-42 amyloid pores in Alzheimer's disease pathology. J Cell Biol. 2011; 195: 515-524.
57. Serra-Batiste M, Ninot-Pedrosa M, Bayoumi M, Gairi M, Maglia G, Carulla N. Aβ42 assembles into specific β-barrel pore-forming oligomers in membrane-mimicking environments. Proc Natl Acad Sci. 2016; 113: 10866-10871.
58. Bode DC, Baker MD, Viles JH. Ion channel formation by amyloid-β_{42} oligomers but not amyloid-β_{40} in cellular membranes. J Biol Chem. 2017; 292: 1404-1413.
59. Di Scala C, Chahinian H, Yahi N, Garmy N, Fantini J. Interaction of Alzheimer's β-amyloid peptides with cholesterol: Mechanistic insights into amyloid pore formation. Biochemistry. 2014; 53: 4489-4502.
60. Mirza Z, Beg MA. Possible molecular interactions of bexarotene – a retinoid drug and Alzheimer's Aβ peptide: A docking study. Curr Alzheimer Res. 2017; 14: 327-334.
61. Huy PDQ, Thai NQ, Bednarikova Z, Phuc LH, Linh HQ, Gazova Z, et al. Bexarotene does not clear amyloid beta plaques but delays fibril growth: Molecular mechanisms. ACS Chem Neurosci. 2017; 8: 1960-1969.
62. Fantini J, Di Scala C, Yahi N, Troadec JD, Sadelli K, Chahinian H, et al. Bexarotene blocks calcium-permeable ion channels formed by neurotoxic Alzheimer's β-amyloid peptides. ACS Chem Neurosci. 2014; 5: 216-224.
63. Casali BT, Reed-Geaghan EG, Landreth GE. Nuclear receptor agonist-driven modification of inflammation and amyloid pathology enhances and sustains cognitive improvements in a mouse model of Alzheimer's disease. J Neuroinflamm. 2018; 15: 43.
64. Tu L, Yang XL, Zhang Q, Wang Q, Tian T, Liu D, et al. Bexarotene attenuates early brain injury via inhibiting microglia activation through PPARγ after experimental subarachnoid hemorrhage. Neurol Res. 2018; 40: 702-708.

65. Dheer Y, Chitranshi N, Gupta V, Abbasi M, Mirzaei M, You Y, et al. Bexarotene modulates retinoid-X-receptor expression and is protective against neurotoxic endoplasmic reticulum stress response and apoptotic pathway activation. Mol Neurobiol. 2018; 55: 9043-9056.
66. Kamp F, Scheidt HA, Winkler E, Basset G, Heinel H, Hutchison JM, et al. Bexarotene binds to the amyloid precursor protein transmembrane domain, alters its α-helical conformation, and inhibits γ-secretase nonselectivity in liposomes. ACS Chem Neurosci. 2018; 9: 1702-1713.
67. Serra-Batiste M, Tolchard J, Giusti F, Zoonens M, Carulla N. Stabilization of a membrane-associated amyloid-β oligomer for its validation in Alzheimer's disease. Front Mol Biosci. 2018; 5: 38.
68. Xiang N, Lyu Y, Zhu X, Narsimhan G. Investigation of the interaction of amyloid-β peptide (11-42) oligomers with a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane using molecular dynamics simulation. Phys Chem Chem Phys. 2018; 20: 6817-6829.
69. Habchi J, Chia S, Galvagnion C, Michaels TCT, Bellaiche MMJ, Ruggeri FS, et al. Cholesterol catalyses Aβ42 aggregation through a heterogeneous nucleation pathway in the presence of lipid membranes. Nat Chem. 2018; 10: 673-683.
70. Bowman GL, Dayon L, Kirkland R, Wojcik J, Peyratout G, Severin IC, et al. Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults. Alzheimer Dement. 2018; 14: 1640-1650.
71. Wang H, Golob EJ, Su MY. Vascular volume and blood-brain barrier permeability measured by dynamic contrast enhanced MRI in hippocampus and cerebellum of patients with MCI and normal controls. J Magn Reson Imaging. 2006; 24: 695-700.
72. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron. 2015; 85: 296-302.
73. Yuan C, Guo X, Zhou Q, Du F, Jiang W, Zhou X, et al. OAB-14, a bexarotene derivative, improves Alzheimer's disease-related pathologies and cognitive impairments by increasing β-amyloid clearance in APP/PS1 mice. Biochim Biophys Acta – Mol Basis Dis. 2019; 1865: 161-180.
74. Chia S, Habchi J, Michaels TCT, Cohen SIA, Linse S, Dobson CM, et al. SAR by kinetics for drug discovery in protein misfolding diseases. Proc Natl Acad Sci USA. 2018; 115: 10245-10250.
75. Habchi J, Chia S, Limbocker R, Mannini B, Ahn M, Perni M, et al. Systematic development of small molecules to inhibit specific microscopic steps of Aβ42 aggregation in Alzheimer's disease. Proc Natl Acad Sci USA. 2017; 114: E200-E208.
76. Schreihofer DA, Oppong-Gyebi A. Genistein: Mechanisms of action for a pleiotropic neuroprotective agent in stroke. Nutr Neurosci. 2019; 22: 375-391.
77. Wang YX, Xia ZH, Jiang X, Li LX, An D, Wang HG, et al. Genistein inhibits $A\beta_{25-35}$-induced neuronal death with changes in the electrophysiological properties of voltage-gated sodium and potassium channels. Cell Mol Neurobiol. 2019; 39: 809-822.
78. Wang YX, Tian K, He CC, Ma XL, Zhang F, Wang HG, et al. Genistein inhibits hypoxia, ischemic-induced death, and apoptosis in PC12 cells. Environ Toxicol Pharmacol. 2017; 50: 227-233.
79. Ma XL, Zhang F, Wang YX, He CC, Tian K, Wang HG, et al. Genistein inhibition of OGD-induced brain neuron death correlates with its modulation of apoptosis, voltage-gated potassium and sodium currents and glutamate signal pathway. Chem Biol Interact. 2016; 254: 73-82.
80. Ren B, Liu Y, Zhang Y, Cai Y, Gong X, Chang Y, et al. Genistein: A dual inhibitor of both amyloid β and human islet amylin peptides. ACS Chem Neurosci. 2018; 9: 1215-1224.

81. Yang Y, Jiang W, Wang L, Zhang ZB, Si SY, Hong B. Characterization of the isoflavone pratensein as a novel transcriptional up-regulator of scavenger receptor class B type I in HepG2 cells. Biol Pharm Bull. 2009; 32: 1289-1294.
82. Eto M, Hashimoto T, Shimizu T, Iwatsubo T. Characterization of the unique in vitro effects of unsaturated fatty acids on the formation of amyloid β fibrils. PLoS ONE. 2019; 14: e0219465.
83. Vela S, Sainz N, Moreno-Aliaga MJ, Solas M, Ramirez MJ. DHA selectively protects SAMP-8-associated cognitive deficits through inhibition of JNK. Mol Neurobiol. 2019; 56: 1618-1627.
84. El Shatshat A, Pham AT, Rao PPN. Interactions of polyunsaturated fatty acids with amyloid peptides Aβ40 and Aβ42. Arch Biochem Biophys. 2019; 663: 34-43.
85. Lee BY, Attwood SJ, Turnbull S, Leonenko Z. Effect of varying concentrations of docosahexaenoic acid on amyloid beta (1-42) aggregation: An atomic force microscopy study. Molecules. 2018; 23: E3089.
86. Fanaee-Danesh E, Gali CC, Tadic J, Zandl-Lang M, Kober AC, Agujetas VC, et al. Astaxanthin exerts protective effects similar to bexarotene in Alzheimer's disease by modulating amyloid-beta and cholesterol homeostasis in blood-brain barrier endothelial cells. Biochim Biophys Acta – Mol Basis Dis. 2019; 1865: 2224-2245.
87. Shrivastav A, Kim HY, Kim YR. Advances in the applications of polyhydroxyalkanoate nanoparticles for novel drug delivery system. Biomed Res Intl. 2013; 2013: 581684.
88. Mamelak M. Energy and the Alzheimer brain. Neurosci Biobehav Rev. 2017; 75: 297-313.
89. Bredesen DE. Reversal of cognitive decline: A novel therapeutic program. Aging (Albany, NY). 2014; 6: 707–717.