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SUBJECT OF PETITION:

Evidence-Based Qualified Health Claim that a
Nutraceutical Formulation (Brand Name Perceptiv[®]) Can
Reduce the Risk of Alzheimer's Disease and Cognitive
Decline During Aging

SUBMITTED TO:

Food and Drug Administration
Office of Nutrition and Food Labeling (HFS-830)
5001 Campus Drive
College Park, MD 20740-3835

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**Petition to the FDA for Authorization of
Evidence-Based Qualified Health Claim that a Nutraceutical Formulation
(Brand Name Perceptiv[®]) Can Reduce the Risk of Alzheimer's Disease
and Cognitive Decline During Aging**

I. INTRODUCTION

A. Preamble

To provide the appropriate background and justification for making the Qualified Health Claim that the Perceptiv formulation can reduce the risk of Alzheimer's disease (AD) and other related claims, it is necessary to review the literature demonstrating the underlying neuropathological events that eventually lead to manifestation of AD, including studies demonstrating that these neuropathological mechanisms can be present for years to decades prior to cognitive decline. It is further necessary to review Mild Cognitive Impairment (MCI), which involves cognitive decline beyond that anticipated for one's age but not yet interfering with general daily tasks, since 40-60% of individuals diagnosed with MCI progress to AD.

Establishment of a Substance-Disease Relationship between the Perceptiv formulation (substance) and Alzheimer's disease (the disease), which encompasses identifying the brain as the affected organ and cognitive decline as the surrogate measure, necessitates a review of the literature demonstrating that nutrition, including the ingredients of the Perceptiv formulation, can reduce the risk of cognitive decline and reduce the deleterious impact of all known underlying neuropathological mechanisms. There are multiple known risk factors for AD, involving multiple, intersecting metabolic pathways; to facilitate review, this petition encompasses a degree of repetition to highlight the interaction of the many identified contributors to the risk of AD.

We further review the literature demonstrating that cognitive difficulties remain the reason one seeks medical attention and further remain as the singular diagnostic criterion during an individual's lifetime. Moreover, since the appropriate biomarkers for a definitive diagnosis of AD can only be obtained following autopsy, a critical degree of cognitive decline, assessed using cognitive tests as described herein, allows only the provisional diagnosis of "probable Alzheimer's" or "senile dementia of the Alzheimer type." While such diagnoses are usually confirmed via autopsy, we demonstrate that therapeutic approaches are ultimately based entirely on maintaining cognitive performance during one's lifetime. In this regard, mitigation of some prominent biomarkers of AD unexpectedly did not delay or reduce cognitive decline.

Clinical studies carried out by myself and my research team are confined to cognitive performance (and associated behavioral complications) and demonstrate that the Perceptiv formulation can improve cognitive performance for individuals with no known or suspected cognitive difficulties, individuals diagnosed with MCI, and for individuals diagnosed with AD. As described herein, our clinical studies not only support that the Perceptiv formulation reduces the risk of AD (since diagnosis is based entirely on cognitive performance and behavioral complications), but further indicate that the Perceptiv formulation exerts beneficial effects for cognitive performance for individuals diagnosed with AD by the same mechanisms as it does for those diagnosed with MCI or with no known or suspected cognitive difficulties.

B. Compliance of Supportive Studies with FDA Specific Requirements for Health Claims

In accordance with Title 21, Subpart E, §101.70 Petitions for Health Claims, and parts 56 (Institutional Review Boards) and 50 (Protection of Human Subjects) <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=56> and <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50>, respectively, the protocols for all clinical studies with the Perceptiv formulation presented herein

were approved by, and carried out in full compliance with, an Institutional Review Board (IRB). Informed consent was required for admission into all studies. For individuals diagnosed with AD, consent of health care proxy and an individual's physician or neurologist was required. Chan et al. (2008a), Chan et al. (2010) and Remington et al. (2009) were approved by the UMass Lowell IRB. Remington et al. (2015a, 2015b and 2016) were approved by the New England IRB (neirb.com); use of an external IRB was mandated by the UMass Lowell Conflicts Committee, who determined that, since UMass Lowell received funding from the Alzheimer's Association to conduct these latter Phase II studies, external IRB approval was required to avoid a conflict of interest. While the overall protocol for each was approved and overseen by the New England IRB, some participating sites in Remington et al. (2015a and 2015b) also were approved by those sites' own IRB. Detailed information regarding all IRB approvals is presented within each publication. Remington et al., 2015a, 2015b and 2016 were registered with ClinicalTrials.gov (NCT01320527 and NCT00903695) as well as the Alzheimer's Association (<http://alz.org/Trialmatch>) since they were funded by the Alzheimer's Association and the Trialmatch site served as a recruitment tool. In accordance with Section 801 of the FDA Amendments Act (<https://www.fda.gov/regulatory-information/food-and-drug-administration-amendments-act-fdaaa-2007/fdaaa-certification-accompany-drug-biological-product-and-device-applications-or-submissions>), our earlier studies (Chan et al., 2008a, 2010; Remington et al., 2009) were not registered with ClinicalTrials.gov since they were Phase I studies; they were not listed on Trialmatch since they were not affiliated with the Alzheimer's Association. All de-identified data from all studies are available if desired. These studies are included separately in Appendix E as well as in the overall reference list (Appendix L) and full collection of references.

All non-clinical studies from my research team were conducted with good laboratory practice regulations as specified in Title 21, Subpart E, §101.70 Petitions for health claims, parts 58 (<https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58>). All studies using animals were conducted under protocols approved by UMass Lowell's Office of Laboratory Animal Welfare-assured Institutional Animal Care and Use Committee (IACUC). Primary cultures were generated following sacrifice of animals in accordance with an IACUC-approved protocol. Analyses of samples from animals, primary cultures, continuous cultures and purified proteins were conducted under good laboratory practice regulations in laboratories approved by UMass Lowell's Institutional Biosafety and Environmental Health and Safety Committees. Non-clinical studies using components of the Perceptiv formulation conducted by my, the Petitioner's, research team are highlighted in **bold** in Appendix L.

All other references were from peer-reviewed journals, with the exception of two summary public reports (one from the U.S. Alzheimer's Association and one from the U.K. Alzheimer's Association and an FDA document). Scrutiny of these references indicates, to the best of the Petitioner's knowledge, that all clinical studies were conducted in accord with the requirements stated in 21 CFR 101.70 (c) and (d) and that all non-clinical laboratory studies were conducted in accordance with good laboratory practice regulations as specified in 21 CFR part 58.

C. Alzheimer's Disease

Alzheimer's disease (AD) is characterized by progressive loss of memory and cognitive function, eventually accompanied by impairments in language and visual and spatial abilities. Impaired neuronal function also manifests as the so-called "Behavioral and Psychotic Symptoms of Dementia" (BPSD), which include depression, apathy, loss of focus or task initiation, and aggressiveness (Silva et al., 2019; De Ture and Dickson, 2019; Chakraborty et al., 2019). An estimated 5.1 million aged Americans have AD, and an additional approximately 200,000 have "Early-Onset" AD (i.e., occurring at <65 years of age). By 2050, the estimated prevalence of AD

in the United States (US) is expected to increase to 11-16 million (Alzheimer's Association Report, 2016; World Alzheimer Report, 2016) and to over 100 million world-wide (Prince et al., 2013).

AD is a multi-faceted, progressive neurodegenerative disorder with no nullifying treatment or cure, and represents the major cause of dementia, affecting nearly 50 million people worldwide (Alzheimer's Association Report, 2016; World Alzheimer Report, 2016; Cummings et al., 2019a; Sloane et al., 2002). To clarify, dementia is a syndrome consisting of a group of symptoms and has a number of causes. AD, by contrast, is a degenerative brain disease and accounts for 60-80% of all cases of dementia¹. AD brings on a relentless decline in an affected individual's ability to carry out daily activities necessitates ever-increasing, and eventually full-time, care, extending the burden to family, and ultimately institutional, caregivers. Total health care costs are expected to reach over \$1 trillion by 2050 (Stefannaci, 2011), with the number of affected individuals more than doubling by this time (World Alzheimer Report, 2016).

There remains an inherent difficulty in diagnosis of AD. A definitive diagnosis of AD requires a critical degree of cognitive impairment as ascertained on standard cognitive tests, coupled with the positive identification of certain pathological hallmarks, specifically extracellular amyloid deposits ("senile plaques") within brain parenchyma and, usually, inter-neuronal "neurofibrillary tangles"; the genesis and impact of these pathological hallmarks will be discussed in detail below. Notably, however, since identification of these hallmarks requires autopsy, manifestation of critical decline in cognitive performance, accompanied by BPSD, fosters only the limited diagnosis of "probable AD" or "senile dementia of the Alzheimer type" pending postmortem examination (McKhann et al., 1984; Couto and Millis, 2015).

D. Mild Cognitive Impairment

Mild Cognitive Impairment (MCI) is a condition related to AD, in which an individual displays cognitive decline beyond that anticipated for his/her age that is noticeable to the individual and to family members and friends, but without BPSD or functional impairment of daily activities (Morris, 2012; Langa and Levine, 2014; Croisile et al., 2012; Bruscoli and Lovestone, 2004; Petersen, 2011). This contrasts with criteria for "probable AD" or "dementia of the Alzheimer type," which requires impairment in cognitive domains *plus* interference with the ability to function in usual daily activities (Morris, 2012). It is estimated that 10% and 20% of individuals ≥ 65 years of age meet the criteria for MCI (Petersen, 2011).

Improvements in diagnosis and detection of biomarkers allow detection of "prodromal AD" (when an individual is displaying deterioration of memory consistent with AD but is still functionally independent; Wilson et al., 2011). Accordingly, a diagnosis of MCI is clinically useful to characterize individuals who have a memory deficit that may resemble prodromal AD in the absence of biomarkers or when testing for biomarkers has not been carried out (Dubois et al., 2010; Langa and Levine, 2014).

MCI is heterogeneous and this variance may influence its progression. Individuals with amnesic or multi-domain MCI are more likely to progress to AD; however, all subtypes can do so, and classification among subtypes can display instability over time (Brusse et al., 2006; Cooper et al., 2015; Rosenberg et al., 2011; Summers and Saunders, 2012; Yaffe et al., 2006; Ganguli

¹ https://alz.org/alzheimers-dementia/difference-between-dementia-and-alzheimer-s?WT.mc_id=eneews2019_09_04&utm_source=eneews-aff-87&utm_medium=email&utm_campaign=eneews-2019-09-04&utm_content=homeoffice&utm_term=Story1. Last accessed 11/16/2019

et al., 2011). Subjective memory decline is a harbinger of greater rates of clinical progression in preclinical AD (Buckley et al., 2016; Geerlings et al., 1999).

Individuals can be further characterized as having “Early MCI” versus “Late MCI” based on subtle differences in performance on standard cognitive tests. An individual is characterized as having Early MCI with performance of 1 to 1.5 standard deviations below the normative mean and as having Late MCI with performance of \geq 1.5 standard deviations below the normative mean (Jessen et al., 2014). As might be anticipated, individuals with Late MCI have a higher risk of conversion to AD than those with Early MCI. In addition, individuals with normal performance on cognitive tests but who self-reported subjective memory concerns had the same risk of onset of AD as those with Early MCI (Jessen et al., 2014).

Notably, it is not inevitable that an individual diagnosed with MCI will progress to AD (Bruscoli and Lovestone, 2004). This may be a function of lifespan, in that a very slow progression may not manifest during an individual’s lifetime. Along the same line of reasoning, while the incidence of AD increases dramatically with age, not all adults present AD but might have done so had their lifespan been significantly longer.

E. Alzheimer’s Disease Consists of Multiple, Inter-related Pathologies

Multiple risk factors for AD have been identified and include age, family history, genotype, diabetes, hypertension, obesity, hypercholesterolemia, brain injury, and low education level (Cummings et al., 2019a; Silva et al., 2019; Vonk et al., 2019). AD is a complex polygenic disease that involves multiple pathologies in several molecular pathways and multiple aberrant interactions among them (Alexiou et al., 2019; Kapasi et al., 2017; De Ture and Dickson, 2019; Mucke, 2009; Teijido and Cacabelos, 2018). Historically, there have been several prevailing theories regarding the initiating and/or central pathological feature, which we will discuss herein.

- Extracellular amyloid deposits (“senile plaques”), which accumulate within brain parenchyma, are composed of beta-amyloid (“Abeta”), which is generated by cleavage of the transmembrane Amyloid Precursor Protein (“APP”) by beta-secretase followed by gamma-secretase. Abeta monomers progressively aggregate into oligomers, fibrils, and eventually insoluble plaques (Selkoe and Hardy, 2016).

- Interneuronal neurofibrillary tangles, another hallmark protein aggregate in AD, are comprised of the microtubule-associated protein tau. Critical phosphorylation events foster extension of the molecule, dissociation from microtubules and formation of paired helical filaments, which accumulate as so-called neurofibrillary tangles (Iqbal et al., 2005).

- Multiple genetic risk factors for AD have been identified (reviewed in Silva et al., 2019) as follows:

Presence of one or two copies of the E4 allele of the APOE gene are strongest genetic risk factor for AD (Hersi et al., 2017). The presence of ApoE4 promotes Abeta accumulation and aggregation, which in turn promotes hyperphosphorylation of tau leading to neurofibrillary tangles. ApoE4 also alters lipid metabolism and oxidative damage (Cacabelos et al., 2012; Corder et al., 1993; Strittmatter et al., 1993; Mattsson et al., 2018; Kamboh, 2004).

Mutations in genes encoding presenilin 1 and 2 are strongly associated with an increased risk of AD (Kelleher and Shen, 2017; Karch and Goate, 2015). Like ApoE4, key mutations in presenilin trigger increased Abeta-mediated pathology, including increased activity of gamma-secretase and resultant increased levels of Abeta (Fuso et al., 2011a, 2011b, 2012; Chan and

Shea, 2007a; Chan et al., 2008b, 2009a). Furthermore, one polymorphism in the gene encoding presenilin-1 is associated with a 10-fold increase in risk of AD for individuals harboring ApoE4 (Benitez et al., 2013). The degree of influence of presenilin-1 on multiple downstream events have led to its characterization as causal rather than consequential (Fuso et al., 2012).

Mutations in genes encoding presenilin 1 and 2, and in the amyloid precursor protein *APP* itself, are associated with early-onset autosomal-dominant AD, also referred to as Familial AD (FAD; Ringman et al., 2014). In this regard, AD in the absence of any known family history is referred to as Sporadic AD (SAD). SAD is more common, and typically has a later onset than FAD with respect to age (Ringman et al., 2014). FAD and SAD, however, display commonalities in neuropathology (Lippa et al., 1996).

Mutations in tau are relatively rare in AD vs. mutations in other contributors and, when observed, are typically associated with FAD rather than SAD (Poorkaj et al., 2001). By contrast, tau mutations are associated with dementia (Sobrido et al., 2003).

As will be described below, most if not all of these at-risk genes are subject to misregulation and resultant overexpression when their respective promoters are insufficiently methylated. Accordingly, both the presence of key mutations and their relative expression level can increase the risk of AD.

More recent technological advances in genome screening have identified multiple additional genes that are associated with an increase in the risk of AD; the relative impact of, and pathogenic mechanisms induced by, these novel candidate genes will require further study (Karch and Goate, 2015; Silva et al., 2019).

•Oxidative stress increases during aging and neurodegeneration, during which mitochondrial failure produces reactive oxygen species (ROS). If ROS are not quenched by endogenous antioxidant systems such as glutathione (and/or consumption of antioxidants), they damage lipids (compromising membrane integrity), proteins (leading to loss of function), and nucleic acid (compromising genetic expression), which can ultimately cause neuronal death (Angelove and Abramov, 2018; McIntosh et al., 1997; Pena-Bautista et al., 2019).

A decrease in levels of glutathione represents an early event in neurodegeneration (Morozova et al., 2007), which contributes to oxidative damage induced by Abeta and potentiated by homocysteine (Ho et al., 2001). Activity of glutathione peroxidase (one of the enzymes that mediates quenching of oxidative species by glutathione) is essential for protection against oxidative damage to lipids in cells and in brain tissue from individuals diagnosed with AD (Yoo et al., 2010).

•The distribution of certain biometals is altered in AD and can exacerbate oxidative damage. Significant alterations in copper levels and localization are observed in brain tissue of individuals with AD: copper *accumulates* within amyloid deposits, while *overall* levels of intracellular copper are deficient (Maynard et al., 2005). Interaction of Abeta with copper can generate increased reactive oxygen species by inhibition of detoxification by copper-dependent SuperOxide Dismutase-1, while overall copper deficiency can promote inflammation (Mathys and White, 2017). Resultant increased oxidative stress can promote increased Abeta accumulation in a deleterious feedback cycle (Bayer et al., 2006; Tonnies and Trushina, 2017). The reductionist approach of primary culture afforded the demonstration that Abeta directly impaired synaptic signaling in cultured hippocampal and mixed cortico-hippocampal cultures and that homocysteine and environmental contaminants including iron and carbon nanoparticles augmented this

impairment (Lee et al., 2015; Taddeo et al., 2014; Oyabu et al., 2019; Tonnies and Trushina, 2017). Direct interactions of metals with Abeta and tau potentiated their misfolding, aggregation and neurotoxicity (Savelieff et al., 2013).

- Inflammation has been strongly linked to the risk and progression of AD, and may involve multiple feedback loops, thought by some to arise initially due to inflammation of small blood vessels within brain tissue (Heneka et al., 2015; Heppner et al., 2015; Marchesi, 2011; McGrattan et al., 2019).

Microglia (the immune cells of the brain) are chronically activated by reactive oxygen species during AD, which can foster neuronal damage by release of the normally neuroprotective inflammatory cytokine nitrous oxide at inappropriate levels (Li et al., 2018; Katsumoto et al., 2018). Increased numbers of activated microglial cells and increased cytokines levels are present in brain tissue from individuals diagnosed with AD (Lee et al., 2010a, 2010b). Microglia are also activated by Abeta and tau burden, which is augmented by environmental factors and diet, while microglia function is compromised by the presence of key mutations in surface receptors, leading to inflammation (Katsumoto et al., 2018). Notably, chronic activity of microglia may play a role in triggering formation of plaques (Li et al., 2018; Spangenberg et al., 2019). There may therefore be feedback between inflammation and Abeta and tau accumulation, augmented by external factors (Heneka et al., 2015; Heppner et al., 2015; Marchesi, 2011; McGrattan et al., 2019; Tonnies and Trushina, 2017).

This inflammatory cascade may also induce astrocyte reactivity, during which astrocytes cease their normal neuroprotective roles, leaving neurons more susceptible to inflammatory damage (Fuller et al., 2010).

- Mitochondrial dysfunction may also contribute to ROS in a deleterious feedback cycle (Rigotto and Basso, 2019; Tonnies and Trushina, 2017). Mice expressing mutant human presenilin 2 and mutant human APP display decreased Nicotinamide adenine dinucleotide phosphate, leading to reduced antioxidant potential (Theurey et al., 2019). Along with increased ROS in cortical tissue of individuals with AD, multiple mitochondrial anomalies are observed, including suppression of axonal transport of mitochondria, and diminished function of those that do reach synapses (McIntosh et al., 1997; Riemer and Kins, 2013; Reddy et al., 2018). Mitochondrial DNA displays increased degradation and deletion in AD (Corral-Debrinski et al., 1994; Krishnan et al., 2012). This could underly energy depletion in neurons, since respiration rate is critically dependent upon mitochondria's own DNA (Rocher et al., 2008).

- Progressive loss of cholinergic neurons, which play central roles in cognition and consequent loss of signaling, is considered to contribute to short-term memory loss in AD (Mufson et al., 2008; Hampel et al., 2018). Prior to actual loss of neurons, there is a progressive decline in the neurotransmitter acetylcholine. Choline is the precursor of acetylcholine and directly influences acetylcholine synthesis in brain tissue (Cohen and Wurtman, 1975). Choline is also a precursor for phosphatidylcholine, which is required for membrane synthesis and maintenance of synaptic function. In this regard, meta-analysis demonstrates that individuals with AD have reduced choline in plasma and cerebrospinal fluid (De Wilde et al., 2017).

- Synaptic dysfunction and excitatory/inhibitory transmitter imbalance are implicated in AD (Li et al., 2017). Some of this may be causal while some may be a result of neuronal damage due to one or more of the other potential causes listed herein.

- Cortical glucose metabolism is diminished in early AD and can precede other abnormalities

(Haxby et al., 1986).

•Depression, common in individuals diagnosed with AD, can negatively influence overall outcomes and is associated with the ultimate need for institutionalization (Ortega et al., 2017; Chakraborty et al., 2019). Depression during early adult years increases the risk of AD during elderly years (Silva et al., 2019). Multiple cognitive impairments are associated with depression including episodic, verbal and working memory, and executive function (Hammar, 2009). Symptoms of depression increased cognitive decline for individuals diagnosed with AD (Zverova et al., 2013). Individuals diagnosed with major depression displayed increased hippocampal atrophy and Abeta deposition (Wu et al., 2018). Depression is also common for individuals diagnosed with MCI and increases the risk of progression to AD (Modrego and Ferrandez, 2004).

With particular relevance to this document, certain nutritional deficiencies are one of the risk factors for developing MCI and progression to AD and can augment the above risk factors (Engelborghs et al., 2014; Mi et al., 2013; George et al., 2009; Cao et al., 2015; Gillette-Guyonnet et al., 2013; Monti et al., 2015). These are discussed in detail below.

F. Multiple Hypothesis Have Been Advanced to Account for Alzheimer Pathology

AD pathogenesis involves a complex interplay of genetic, epigenetic, and environmental factors (reviewed in Teijido and Cacabelos, 2018; Yegambaram et al., 2015; Killin et al., 2016; Reitz et al., 2011). Mapping the order of pathological events that accompany AD has been a focus of many investigators and has given rise to several hypothetical sequelae. These potential sequelae apply to the *de novo* diagnosis of AD as well as conversion of a prior diagnosis of MCI into AD.

The “Amyloid Cascade Hypothesis” suggests that Abeta accumulation is pivotal in that it perturbs synaptic and overall neuronal function, fosters accumulation of tau and formation of neurofibrillary tangles, and ultimately contributes to sequential neuronal loss (Selkoe and Hardy, 2016). In support of this hypothesis, a great number of mutations in presenilin 1, 2, and APP that can cause early-onset familial AD all increase Abeta toxicity, strongly supporting a pivotal role for Abeta in AD pathology. Some but not all studies indicated poorer clinical prognosis and faster decline in episodic memory among cognitively normal adults that have an increased Abeta burden (Buckley et al., 2016; Hollands et al., 2015; Lim et al., 2013; Villemagne et al., 2011). Abeta indeed fosters multiple AD-related pathological events: Abeta induces ROS and membrane damage in brains of mice expressing human ApoE4, which have a greater vulnerability to Abeta-induced ROS (Butterfield, 2002). Abeta and hyperphosphorylated tau induce defective autophagy in AD pathogenesis (Reddy and Oliver, 2019; Reddy et al., 2018), perhaps fostering accumulation of toxic protein fragments. Abeta perturbs neuronal signaling (Amar, 2017; Lee et al., 2015; Taddeo et al., 2014). Moreover, subcytotoxic levels of Abeta disrupt complex synaptic signaling patterns in cortico-hippocampal cultures, suggesting that Abeta could exert detrimental effects on processing of information during its initial accumulation that might not yet interfere with daily life but could impair reaction time that may be critical during activities such as driving, etc. (Lee et al., 2015; Taddeo et al., 2014).

Continued advances, however, indicate that this hypothesis fails to explain all aspects of AD (Karran and De Strooper, 2016). For example, while Abeta accumulation is indeed observed in all individuals with AD, it is also observed in the absence of cognitive decline (De Strooper and Karran, 2016; Bennett et al., 2006). Consistent with this was continued cognitive decline in some but not all studies following immunotherapeutic clearance of amyloid from brain tissue of individuals with sporadic AD (Holmes et al. 2008; Tolar et al., 2019). Furthermore, recent analyses

demonstrate that the genetic risk for AD can be distinct from, and may require more than, the genetic risk for amyloid accumulation (Leonenko et al., 2019).

Similar criticism has been advanced against the “Mitochondrial Cascade Hypothesis,” which suggests that mitochondrial dysfunction, as described above, promotes SAD pathology (Swerdlow et al., 2014). However, this hypothesis does not account for the protective influence of a particular mutation in APP that reduces Abeta production and aggregation (Jonsson et al., 2012; Maloney et al., 2014). This does leave open the possibility that mitochondrial dysfunction may promote ROS and inflammation that drives Abeta production but contradicts the possibility that AD is independent of Abeta-induced neuropathology.

The “Modified Amyloid Cascade Hypothesis” highlights the role of tau, postulating that Abeta accumulation accelerates pre-existing tau pathology, and fosters the spread of tau pathology (Karran and De Strooper, 2016). In this regard, oxidative stress can be detected prior to Abeta accumulation, and hyperphosphorylated tau induces oxidative stress, prompting referring to/defining AD as a “tauopathy” (Haque et al., 2019). Neurofibrillary tangle deposition correlates with loss of synapses during AD progression, and each of these processes is correlated with cognitive decline (Zempel and Mandelkow, 2014), but this correlation does not rise to the level of causality. It remains unclear whether the development of tau pathology is antecedent to or parallels that of Abeta (Karran and De Strooper, 2016).

The so-called “Dual Pathway Hypothesis” (Small and Duff, 2008) suggests that upstream factors induce Abeta and tau accumulation, but also foster other pathologies, and as such, prevention (or clearance) of these hallmarks would not be expected to prevent AD progression (De Strooper and Karran, 2016).

The “Cholinergic Hypothesis” is focused on the progressive loss of cholinergic neurons, which are abundant throughout the thalamus, striatum, limbic system, and neocortex, and which play essential roles in learning, memory, and complex brain function (Hampel et al., 2018). Progressive loss of these neurons is considered to underlie development and progression of AD. (Hampel et al., 2018).

The “Vascular Hypothesis” derives from observation of progressive disorganization of the capillary network in brain tissue of individuals with AD, ultimately resulting in an overall reduced vascular network (Karran and De Strooper, 2016). Notably, in addition to accumulation of Abeta in plaques, Abeta often surrounds capillaries. It has been suggested that AD is initiated as a result of damage to small blood vessels by oxidatively-induced inflammation and Abeta accumulation (Marchesi, 2011). It remains unclear, however, whether vascular deficiency or damage fails to clear Abeta prior to aggregation, whether Abeta aggregation induces vascular damage, or whether both occur in a deleterious feedback mechanism.

The “Metabolism Hypothesis” is derived from the observation that insulin signaling, which is essential for synaptic maintenance, is impaired in brains of individuals with AD. This prompted the novel definition of AD as “Type 3 Diabetes” (Steen et al., 2005). However, as with other hypothesis, it remains unclear as to whether abnormalities with insulin signaling pathways are upstream of, or are themselves a result of, other pathological events in AD (Karran and De Strooper, 2016).

The “Cell-Cycle Hypothesis” was derived from the observation of increased expression of mitotic kinases in brains of individuals with AD, the demonstration that antibodies generated with paired helical filaments also reacted with epitopes in dividing cells, and that expression of

oncogenes in differentiated (and by definition therefore post-mitotic) neurons induced DNA duplication and increased abnormally phosphorylated tau but did not induce division. These findings prompted the consideration that a futile attempt to re-enter the mitotic pathway fostered tau pathology and neuronal death (Karran and De Strooper, 2016).

Multiple interactions can be traced among all of the above pathological events, rendering it difficult if not impossible to define the initial cause. In this regard, there may be no singular, universal cause. It remains possible that, whatever the order of pathological events in AD, there could be multiple entry points into the cascade. This is highlighted by the number of mutations of different proteins that increase the risk of AD. Accordingly, AD pathology is complex and is comprised of multiple dynamic components, and the events described in all of the above hypotheses may interact simultaneously and/or sequentially (Selkoe and Hardy, 2016; Mizuno et al., 2012; Reitz et al., 2011; De Strooper and Karran, 2016; Tonties and Trushina, 2017).

G. Novel Diagnostic Approaches for Alzheimer's Disease and Mild Cognitive Impairment

Diagnostic approaches for AD and MCI are advancing rapidly and provide insight into pathological hallmarks previously only accessible during autopsy. Novel imaging methodologies and analyses of cerebrospinal fluid reveal that accumulation of Alzheimer-specific biomarkers (e.g., aberrant levels of beta-amyloid and abnormal forms of tau) can precede any cognitive indications by 10 years or more (De Meyer et al., 2010; Grimmer et al., 2015; Wang et al., 2015; Bennett et al., 2006; Di Domenico et al., 2015) and can provide supportive information to accompany diagnosis during cognitive difficulties (Jack et al., 2018; Niemantsverdriet et al., 2017). These findings are critical, since (1) individuals in which imaging studies demonstrate aberrant levels of Abeta in a pre-dementia state declined faster than those with normal levels (Papp et al., 2016; Petersen et al., 2016), and (2) imaging and analysis of cerebrospinal fluid, coupled with analysis for at-risk genes, can also assist with prediction of conversion from MCI to AD (Grimmer et al., 2013, 2015; Hatashita and Yamasaki, 2013; Jannsen et al., 2015; Petersen et al., 2010; Lehalier et al., 2015; Nesteruk et al., 2015; Hatashita and Yamasaki, 2013; Okello et al., 2009; Handels et al., 2017; Wirz et al., 2014; Zhang et al., 2019b; Nabers et al., 2018).

Multiple recent studies indicate that plasma Abeta levels correlate with those of cerebrospinal fluid, which would provide an inexpensive, non-invasive test that could predict cerebral amyloid deposition and potential increased risk for AD (Nakamura et al., 2018; Hanon et al., 2018; Fandos et al., 2017). Baroni and colleagues (2019) suggest, based on a positive correlation of folate with cognitive performance, that monitoring plasma B vitamin status among the elderly is an economic and practical approach for assessment of cognitive decline and possible early intervention. Use of plasma for detection of biomarkers would be ideal, since it is substantially less expensive than imaging and substantially less invasive than a spinal tap to obtain cerebrospinal fluid. Abeta, tau, homocysteine, oxidatively-damaged lipids and even differential microRNAs can be detected in plasma (Zhang et al., 2019a). Unfortunately, comprehensive analyses of published reports on quantification of Abeta and tau in plasma have yielded contradictory results thus far, and while promising, remain at a stage where they may prompt mis- or overdiagnosis (Lee et al., 2019; Ritchie et al., 2017; Freeman et al., 2016). Similarly, inflammatory biomarkers in plasma may support diagnosis and prediction of clinical outcome in AD and MCI, but further studies are required to substantiate this approach (Morgan et al., 2019). Rather than relying simply on overall Abeta levels in plasma as an indicator, the ratio of Abeta consisting of 40 amino acids versus 42 amino acids (the “40/42 ratio”) has been reported to reflect amyloid deposition in brain tissue of cognitively normal individuals at risk for AD (Vergallo et al., 2019). Microstructural lesions were positively associated with prodromal stages of AD, including Abeta-positive MCI, suggesting that they represent a useful corroborative biomarker. However, they were inconsistent for identifying

individuals with preclinical AD even if such individuals were Abeta-positive (Stefan et al., 2019). Unfortunately, however, mixed pathologies predominate in the older population (which are the segment of the population most at risk for AD) and the associations between biomarkers, neuropathology and clinical syndromes are not clear (Waite, 2015).

Oxidative damage leads to lipid peroxidation. Some lipid peroxidation compounds in plasma were significantly correlated with brain tissue atrophy revealed by neuroimaging in early AD (Pena-Bautista et al., 2019). While the authors indicate that further studies are required, lipid peroxidation in plasma could provide an additional biomarker of early AD. Imaging of amyloid burden, tau, and resting and functional magnetic resonance imaging (MRI) to monitor hippocampal atrophy all hold promise to assist with and improve diagnosis, but these phenomena may not reveal enough change prior to cognitive decline (Marquez and Yassa, 2019; Hampel et al., 2018). Continued developments in imaging incorporating novel cloud-based computer assisted database comparisons holds promise (Sakamoto et al., 2019). Declined attention accompanies MCI and is reflected by subtle changes in visual attention as monitored by electroencephalography (EEG) and by the perspective of eye movement. This suggests that such analyses may be useful for detection of early MCI (Jiang et al., 2019).

Even in combination, however, imaging and biomarkers could not provide an accurate prediction of short-term cognitive decline or conversion from MCI to AD (Ottoy et al., 2019). Moreover, the genetic risk for AD remains distinct from the genetic risk for amyloid accumulation (Leonenko et al., 2019), leaving open the potential for false positives. Indeed, age rather than a diagnosis of AD is the major predictor of Abeta accumulation (Fukumoto et al., 2003).

As discussed in the following section, cognitive decline, either detected by an individual or caregiver, remains the major if not the only reason that warrants consultation with a physician, with imaging and biomarker analyses serving only to corroborate a diagnosis of MCI or AD (i.e., “probable dementia” or “senile dementia of the Alzheimer type”).

What imaging and biomarker studies clearly demonstrate is that not only is the most prominent and clinically-definitive AD biomarker (Abeta) detectable in cognitively-normal adults, but that both Abeta and the strongest genetic risk factor for AD (one or more copies of ApoE4) are associated with diminished cognitive performance among adults still performing within a cognitively normal range (Aizenstein et al., 2008; Ewers et al., 2012; Hollands et al., 2015; Kantarci et al., 2012; Lim et al., 2012; Mormino et al., 2014; Oh et al., 2016; Petersen et al., 2016; Rentz et al., 2010; Rolstad et al., 2011; Sojkova et al., 2011; Sperling et al., 2013; Stomrud et al., 2010; Wirth et al., 2013; Bennett et al., 2006). ApoE4 also accentuated the deleterious influence of Abeta on cognitive function in individuals still performing within a cognitively normal range in some (Kantarci et al., 2012; Stomrud et al., 2010) but not all (Petersen et al., 2016; Lim et al., 2012) studies. *This underscores the continuity between normal aging and AD, and highlights factors that increase the risk of AD.*

Along these lines, cognitive tests demonstrate that some aspects of cognitive decline, including memory, reasoning, spatial visualization, processing speed and executive function, begin when individuals are 20-30 years of age and continue throughout life (Salthouse, 2009; Tombaugh, 2004).

As with many age-related chronic conditions, earlier detection affords the best opportunity for intervention (Shea and Remington, 2015). Pharmacological approaches directed against Abeta or tau for individuals diagnosed with AD have been unsuccessful, leading to the suggestion that treating established disease may be too late (Waite, 2015). Individuals at increased risk as

revealed by these novel approaches may benefit from timely initiation of pro-active lifestyle modifications, including optimizing their nutritional intake and consuming appropriate supplements. *In this regard, the studies presented herein demonstrate that, while the Perceptiv formulation is beneficial even when initiated after the onset of AD, it was most effective when initiated prior to any cognitive decline and for individuals diagnosed with MCI.*

H. Cognitive Decline is the Impetus for Consultation with a Physician

The combination of neuropsychological examination and MRI improved prediction of conversion from MCI to AD versus either approach alone (Salvatore et al., 2018). Accordingly, all of the above technological advances will ultimately augment and improve diagnosis, especially when used in combination (Silva et al., 2019), but cannot be expected to replace or eliminate cognitive performance as a critical diagnostic measure.

This in many cases is unfortunate and delays timely intervention, since AD can be preceded by subtle memory decline that can last a decade or more before progressing to what would be diagnosed as MCI (Caselli et al., 2017). During this early stage of decline, individuals and even their caregivers can fail to perceive any serious difficulty, and therefore will not consult a physician (Koskas et al., 2018).

We observed this during our clinical studies. As will be described in detail in supportive results, a number of elderly participants who self-reported no cognitive difficulties displayed a severe decline in performance on an executive function test over 3 months (Shea and Remington, 2018a). Since these participants self-reported no known nor suspected cognitive decline, their respective ongoing cognitive decline likely did not pervade day-to-day performance. In support of this possibility, these individuals did not display a similar decline on tests assaying simple memory (Chan et al., 2010; Shea and Remington, 2018a).

Many individuals and/or their caregivers may remain unaware, or unwilling to accept, that they are experiencing the early stages of clinical cognitive decline (Barrett et al., 2006; Kotagal et al., 2004; Jessen, 2014; Vannini et al., 2017; Shea and Remington, 2018a, 2019; Krolak-Salmon et al., 2019). This may be augmented by a feeling of hopelessness or denial since there is no cure for AD (Krolak-Salmon et al., 2019). Even if an individual is aware of memory difficulties, it is unclear whether or not assistance will be sought (Begum et al., 2012), especially in the absence of any family history of AD (Fladby et al., 2017). This unfortunately delays intervention, including that of relatively simple lifestyle modifications that could delay (McGough et al., 2017; Shlisky et al., 2017; Knight et al., 2016; Gallagher et al., 2016; Lee and Kim, 2016; Shea et al., 2012; Shea and Remington, 2012a,b; Soldevila-Domenech et al., 2019; Anastasiou et al., 2018) or even reverse (Hertzog et al., 2008; Barekatain et al., 2016) cognitive decline provided they are initiated sufficiently early.

Pending the development or refinement of biomarker analyses that can be easily and inexpensively administered, such as during a routine physical examination, cognitive difficulties will remain the major if not the only reason prompting an individual, or the caregiver(s) of an individual, to seek medical attention (Shea and Remington, 2019). This ideally warrants simple intervention(s) that can improve cognitive performance prior to any decline, as well as improve or at least maintain cognitive performance during clinically-apparent decline including individuals with MCI and AD. Notably, improvement of cognitive performance, rather than simple maintenance, would encourage compliance of individuals who demonstrate no cognitive impairment as well as individuals described above that have declined somewhat but remained unaware of it. Demonstration that the same intervention continues to be effective even following a diagnosis of AD would further encourage continued use by pro-active, cognitively-intact

individuals and, in doing so, delay or reduce the risk of AD. *Studies reviewed in detail below identify the Perceptiv formulation as one such intervention.*

I. Pharmacological Approaches in Management of Alzheimer's Disease

None of the FDA-approved pharmacological treatments for cognitive symptoms of AD provide a cure, but rather delay progression of AD (Cummings et al., 2015, 2019a). Since AD encompasses multiple pathological events as described above, a multi-target combinatorial therapeutic approach may be essential. However, combinatorial approaches with existing pharmacological agents also provide only temporary delay of AD progression, with no restoration of neuronal health (Cummings et al., 2019a; Fessel, 2017; Shah and Reichman, 2006). They are not effective for everyone, and even when effective, they ultimately lose efficacy as the disease progresses and are frequently discontinued due to adverse effects (Birks, 2006; Cummings et al., 2015; Raina et al., 2008). Additional combinatorial therapies are warranted, since cholinergic drugs function only symptomatically and have a limited window of efficacy; as acetylcholine production continues to decline, cholinergic inhibitors eventually lose efficacy (Cummings et al., 2018; Hampel et al., 2018).

While depression is associated with AD and MCI, and progression of MCI to AD, conflicting evidence has been presented on the efficacy of existing antidepressants for cognition and depression for individuals diagnosed with AD (Ortega et al., 2017; Chakraborty et al., 2019; Modrego and Ferrandez, 2004). More analyses are required to determine whether or not antidepressants represent a useful augmentation of therapeutic approaches to mitigate progression of AD (Ortega et al., 2017).

The lack of pharmacological agents that provide disease modification has fostered consideration of other approaches (Waite, 2015).

J. Non-Pharmacological Approaches for Maintenance of Cognitive Function and Reduction of Risk of Alzheimer's Disease

Immunotherapeutic approaches with anti-Abeta antibodies are receiving growing attention. It is likely that soluble Abeta oligomers are the form that induce toxicity and compromise synaptic activity and memory, rather than Abeta accumulated within plaques (Busciglio et al., 1993; Cleary et al., 2005; Lesne et al., 2006; Walsh et al., 2002; Jacobsen et al., 2006). Along these lines, elimination/reduction of generation of Abeta represents a more appropriate therapeutic target than attempting to dissociate existing plaques (e.g., Klyubin et al., 2005). Unfortunately, as described above, individuals with sporadic AD underwent continued cognitive decline following immunotherapeutic clearance of amyloid from brain tissue (Holmes et al. 2008). Notably, in several trials, meningoencephalitis occurred. Multiple recent and ongoing studies utilize monoclonal antibodies, which reduced Abeta levels and did not provoke meningoencephalitis, but no definitive cognitive benefit has been observed (Barrera-Ocampo and Lopera, 2016). By contrast, a monoclonal antibody (aducanumab) directed against Abeta reduced brain levels of Abeta in transgenic mice and individuals with prodromal or mild AD, which was accompanied by a slowing of cognitive decline in Phase I trials (Sevigny et al., 2016). While apparent failure of efficacy led to halt of Phase 3 clinical trials, recent investigation of complete data sets from these trials indicated modest but significant reduction in the rate of certain aspects of cognitive decline for some individuals diagnosed with early-stage AD (Tolar et al., 2019). These results remain controversial and do not establish efficacy (Howard and Liu, 2019) but nevertheless are hopeful. Longer-term trials are warranted and the company (Biogen) is currently seeking FDA approval.

A growing body of literature supports lifestyle modification. Dresden (2014) and colleagues (2016) demonstrated reversal of cognitive decline for a small number of individuals displaying

subjective cognitive impairment, for individuals diagnosed with MCI, and for individuals with early AD following their combinatorial “metabolic enhancement for neurodegeneration (MEND)” approach. This approach was personalized for participants and encompassed elimination of processed foods, adoption of a Mediterranean-style diet enriched in certain foods (fish, fruits, vegetables, nuts), supplementation with B vitamins and vitamin D, and lifestyle alterations including meditation and relaxation to reduce stress. After 5-24 months, individuals, most of whom were ApoE4 positive, displayed improvements in cognitive performance and daily function and in some cases displayed partial restoration of hippocampal volume. Similarly, combinatorial intervention involving dietary modifications along with physical and cognitive training fostered cognitive improvement in elderly individuals who had not displayed cognitive impairment (Ngandu et al., 2015). Dietary modification can reduce inflammation (McGrattan et al., 2019; Gardener et al., 2016), which, as described above, can otherwise generate a deleterious feedback loop involving Abeta and tau deposition, leading to microglial involvement and further inflammation.

Lifestyle modifications including nutritional and social enrichments and cognitive exercise/training can enhance and preserve cognitive performance in older adults (Kelley et al., 2017; Anastasiou et al., 2018; Hersi et al., 2017; Camfield et al., 2011; Monti et al., 2015; Llamas-Velasco et al., 2015; Groot et al., 2015; Hoffmann et al., 2015; Yang et al., 2015; Wollen, 2010). Moreover, multiple studies indicate that improved nutrition promotes and maintains cognitive performance throughout the adult life span (Dauncey, 2014; van de Rest et al., 2015; Shea and Remington, 2015; Shea and Remington, 2018a, b, 2019; Monti et al., 2015). Meta-analysis indicated that physical activity had a moderate to strong pooled Clinical Effect size for MCI and a small Clinical Effect size for AD (Strohle et al., 2015). A longitudinal analysis of >10,000 individuals demonstrated that increased social activity during mid-life reduced the risk of dementia later in life (Sommerlad et al., 2019). Lifestyle modifications have the advantage that they can be initiated at any time, as opposed to the inherent compromise of waiting until sufficient cognitive decline has transpired to warrant diagnosis of AD and prescription of pharmacological agents (Shea and Remington, 2015, 2018a, 2019; Koskas et al., 2018; Waite, 2015).

A multi-component approach including physical exercise, mental exercise, modified diet, anti-inflammatory nutritional supplements, sleep optimization, and stress management resulted in improvement in cognitive performance, daily activities and brain connectivity for individuals diagnosed with varying levels of cognitive impairment. Three of the five patients were no longer classified as cognitively impaired, while a fourth patient improved from moderately-to-severely impaired to mildly impaired (James et al., 2019; Downey et al., 2019). Meta-analysis indicated that physical activity had a moderate to strong pooled Clinical Effect size for MCI and a small clinical effect size for AD (Strohle et al., 2015).

While non-pharmacological interventions can improve cognition and delay progression of MCI to dementia, and in some cases may be as or more effective than current pharmacological approaches, heterogeneity of subjects and variability in interventions and outcomes continue to limit generalizability (Serrano-Pozo and Growdon, 2019; Horr et al., 2015; Fenech, 2017; Liang et al., 2018; Sherman et al., 2017; McMaster et al., 2018; Botchway et al., 2018). Interpretation of non-pharmacological approaches is further confounded since participants are typically not naive to treatments and participants may also have inherent deficiencies in metabolism or differential adsorptive capacity of nutrients (Shea and Remington, 2015). In some cases, combinatorial approaches with non-pharmacological and pharmacological agents resulted in increased efficacy of both components (Shea and Remington, 2015; Cummings et al., 2019a, b).

Shea and Remington (2019) noted that individuals diagnosed with MCI but classified as “intact” did not show improvement following a nutritional intervention (i.e., the Perceptiv

formulation), but maintained their baseline performance, while those classified as “impaired” did display improvement versus placebo and versus their own baseline performance. These findings highlight that lifestyle modifications may in some cases not impart apparent improvement for individuals with initial high-level functioning but may nevertheless be involved in maintenance of existing levels of cognitive performance. *One approach is therefore the establishment and refinement of non-pharmacological lifestyle modifications that can prevent or delay cognitive/behavioral decline associated with AD yet remain benign or helpful to an individual not presenting AD* (Arenaza-Urquijo et al., 2015; Rolandi et al., 2015).

K. Nutritional Approaches for Maintenance of Cognitive Function and Reduction of Risk of Alzheimer’s Disease

A central component in the above lifestyle modification studies is improvement in nutrition. Increased attention has been given to vitamins, supplements and medical foods in efforts to reduce the risk of developing multiple age-related disorders including AD, as well as for treatment of existing disorders (Varteresian and Lavretsky, 2014). Nutrition is one modifiable risk factor that influences the risk of AD (van de Rest et al., 2015; Gillete-Guyonnet et al., 2013; Silva et al., 2019; Barberger-Gateau et al., 2007; Cao et al., 2015; Dominguez and Barbagallo, 2015; Virmani et al., 2013; Wollen, 2010). Adequate nutrition is essential for maintenance of cognitive function during aging, and poor nutrition is one of the risk factors for developing MCI and progression to AD (Engelborghs et al., 2014; Mi et al., 2013; George et al., 2009; Gillete-Guyonnet et al., 2013; Barberger-Gateau et al., 2007; Cao et al., 2015; De Sousa and Amaral, 2012). Up to 30% of dementia in the elderly is attributable to potentially modifiable risk factors (Norton et al., 2014). Approximately 20% of individuals progressing from MCI to dementia do so because of poor diets, diabetes, and other neuropsychiatric symptoms that are potentially preventable (Livingston et al., 2017; Cummings et al., 2019b). Unlike pharmacological approaches, nutritional approaches harbor a low risk for side effects (Mi et al., 2013). Current evidence from animal and human studies suggests that all vitamins influence pathways and pathologies associated with MCI or AD, including those relating to amyloid deposition and tau phosphorylation, DNA damage and repair, mitochondrial function, glucose metabolism, lipid and phospholipid metabolism, neurotransmitter levels and One-Carbon Metabolism (Fenech, 2017). Moreover, B vitamins are cofactors in all of these pathways/pathologies and, along with Vitamin E and C, serve a protective role against dementia (Fenech, 2017).

Shea and Remington (2012a) make the important observation that a confounding factor in attaining any meaningful analyses of potential benefit with a nutritional intervention for reducing the risk of AD is the difficulty of identifying and/or recruiting sufficient numbers of individuals that have low or inadequate baseline nutritional status, such that a comparison can be made among defined initiation of supplementation versus continuing to receive no supplementation. Perhaps the most serious limitation to finding significance in nutritional intervention is that participants are rarely naive to the intervention. Moreover, should participants have routinely consumed the nutritional substance being tested, consuming increasing levels during the test period may not result in improved performance. By contrast, should they have been chronically deficient in the substance prior to testing, they may have already incurred irreversible damage (Shea and Remington, 2012a, 2012b, 2015).

Despite the inherent difficulty of nutritional approaches, several studies have demonstrated the effectiveness of nutritional supplementation. For example, when individuals selected from those with dietary insufficiency in folate were randomized to receive folic acid or placebo for 3 years, slower cognitive decline was observed for those individuals receiving folate (Durga et al., 2007). A separate study demonstrated that folate deficiency may increase risk for MCI and/or probable AD in later life (Agnew-Blais et al., 2015). Folate and vitamin B12 levels in plasma were

significantly lower, while homocysteine levels were higher, for individuals diagnosed with MCI and AD (Ma et al., 2017). Similarly, efficacy of vitamin E supplementation was observed in individuals with low baseline intakes, while those already consuming the recommended daily allowances did not show benefit (Kang et al., 2006, 2008). In this regard, Vitamin E levels are reduced in plasma of individuals diagnosed with AD (Browne et al., 2019).

Consumption of healthy dietary patterns, including the Mediterranean diet with and without combination with the DASH (Dietary Approach to Stop Hypertension) diet and combinations of supplements decreased the risk of onset of AD (Pistollato et al., 2018). A 6-year longitudinal study with nearly 300 individuals with dementia (72% of whom were diagnosed with AD) demonstrated that poor nutrition was associated with faster cognitive decline and more severe functional decline, while improved nutrition had the opposite effects (Sanders et al., 2016). By contrast, in a long-term prospective cohort study, midlife diet was not significantly associated with subsequent risk of dementia. Interactions among nutrients, coupled with the multiple underlying pathologies in AD, render it difficult to assess the impact of supplementation with any single food or nutrient (Akbaraly et al., 2019). Longer follow-up may be required (Akbaraly et al., 2019). The simplest clinical approach is to randomize individuals to a supplement cohort or placebo cohort, with participants sorted according to the degree of dementia at baseline. However, a considerable number of additional randomized cohorts may be necessary. An individual's nutrition and supplement intake prior to the start of the test should be considered, and participants should be grouped accordingly (Shea and Remington, 2012a, 2012b). Even if these conditions were met, the diet among participants will vary, and likely introduce discrepant relative amounts of vitamins and nutrients derived from food. As pointed out by Morris and Tangney (2011), in the absence of such additional stratification, studies of vitamin therapy for the risk of AD harbor a potentially fatal compromise at the outset. Notably, pharmacological interventions are substantially less subject to these complications, with the potential exception of an increased-dose drug test.

There are further potentially confounding factors: It is to be expected that consumption of a complete, healthy diet, coupled with supplementation of key vitamins will help maintain overall health including promotion of cognitive performance. Unfortunately, individuals with AD are at risk of nutritional insufficiencies because of physiological and psychological factors (De Wilde et al., 2017). Meta-analysis demonstrated lower levels of folate, vitamin B₁₂, E, A and C, choline as phosphatidylcholine, docosahexaenoic acid, eicosapentaenoic acid and selenium in plasma, and reduced levels of folate, vitamin B₁₂, E, C, and vitamin (DHA), choline-containing lipids and docosahexaenoic acid in both cerebral spinal fluid and brain tissue, of individuals diagnosed with AD versus cognitively intact elderly individuals (De Wilde et al., 2017). The investigators highlight that brain tissue is critically dependent upon nutrient supply from systemic circulation and suggest that individuals with AD may have specific nutritional requirements that may be met by a multicomponent nutritional intervention. It should be considered that the recommended daily allowances for general health may be insufficient to reduce the risk of onset or progression of AD (Shea and Remington, 2015). This is underscored by the efficacy of a high dose of vitamin E for activities of daily living in AD and progression of AD (Dysken et al., 2014; Sano et al., 1997). Further along these lines, although folate supplementation curtailed oxidative damage in otherwise untreated cultured neurons, a five-fold increase in the normal levels of folate was required to quench oxidative damage induced by exogenous Abeta (Dhitavat et al., 2005). Notably, plasma levels of homocysteine alone do not necessarily indicate alterations of S-adenosyl methionine or expression of Presenilin-1 as quantified in cerebral spinal fluid of individuals diagnosed with AD (Mulder et al., 2005), which is consistent with disparities between plasma and spinal fluid content of other AD biomarkers (Lee et al., 2019; Ritchie et al., 2017) Similarly, increased plasma levels of homocysteine in individuals diagnosed with AD or MCI did not correlate with reduced folate and B12 in one study (Ariogul et al., 2005).

Evidence that any single nutrient has a beneficial effect on cognition or lowers the risk for AD is limited, but this is to be expected given the multiple neuropathologies and neuronal compromises observed during aging and AD (Engelborghs et al., 2014; Gillete-Guyonnet et al., 2013). Just as a multi-target approach is likely to be more beneficial with pharmacological approaches (Cummings et al., 2018), a multi-target approach using combinatorial formulations of nutraceuticals is more likely to have a positive impact (Engelborghs et al., 2014; George et al., 2009; Shea and Remington, 2012a,b; Gillete-Guyonnet et al., 2013; Cummings et al., 2019a,b; van de Rest et al., 2015; Pistollato et al., 2018).

Epidemiological research highlights that dietary supplementation lowers the risk of developing dementia including AD, including but not limited to supplementation with B vitamins involved in homocysteine metabolism, the anti-oxidant vitamins C and E, flavonoids, polyunsaturated omega-3 fatty acids, vitamin D and macronutrients (Gillete-Guyonnet et al., 2013; Famenini et al., 2017; Lee et al., 2016). Meta-analysis showed that omega-3 supplementation improved attention and processing speed for individuals diagnosed with MCI. However, individuals who were cognitively normal or who had been diagnosed with AD did not display any benefit (Mazereeuw et al., 2012) nor did supplementation reduce the risk of AD (Sydenham et al., 2012). Multiple prospective studies, cross-sectional studies and longitudinal studies indicated that a Mediterranean-type diet is associated with slower cognitive decline, a reduction in progression from MCI to AD, a reduced risk of AD, and a decrease in mortality in AD patients (Solfirizzi et al., 2011; van de Rest et al., 2015; McGrattan et al., 2019). The Mediterranean diet can also reduce inflammation (McGrattan et al., 2019). Levels of the endogenous antioxidant glutathione are influenced by overall diet (Gould and Pazdro, 2019).

Epidemiological studies indicate that consumption of fruits and vegetables is associated with improved cognitive performance in aged individuals and with a reduction in risk of neurodegenerative disorders (Letenneur et al., 2007; Joseph et al., 2013). Consumption of blueberries improved memory in older adults (Krikorian et al., 2010a). Grape juice improved cognitive performance for individuals diagnosed with MCI or AD, while apple juice improved mood for individuals diagnosed with AD (Krikorian et al., 2010b, 2012; Remington et al., 2010). The mechanism(s) behind these effects were examined in mouse models and in culture. Grape juice improved cognitive performance in mice (Krikorian et al., 2010b). Apple juice attenuated oxidative stress, presenilin-1 overexpression, Abeta levels and prevented the decline in acetylcholine levels and cognitive performance following dietary or genetically-induced oxidative stress in mice (Chan and Shea, 2006b, 2009c; Rogers et al., 2004; Tchantchou et al., 2005b). Apple juice also prevented Abeta-induced oxidative damage in culture (Ortiz and Shea, 2004).

Two medical foods, Souvenaid and Cerefolin-NAC, report efficacy for cognitive performance in AD. Souvenaid (also known as Fortasyn Connect; a medical food containing folic acid, vitamins B12, B6, C, and E, selenium, uridine monophosphate, docosahexaenoic acid, eicosapentaenoic acid, choline, and phospholipids) had clinical cognitive benefit and reduced plasma homocysteine for individuals diagnosed with early, but not mild-moderate, AD (reviewed in Cummings et al., 2017; Rasmussen, 2019; Engelborghs et al., 2014; Mi et al., 2013). Accordingly, individuals diagnosed with MCI may continue to benefit from Souvenaid should they progress to early stages of AD; unfortunately, however, this formulation is not recommended for individuals who have progressed to moderate or severe AD (Scheltens et al., 2010, 2012; Shah et al., 2013). Cerefolin-NAC (Vitamin B12, L-methylfolate and N-acetylcysteine) is approved for the treatment and prevention of certain vitamin deficiencies seen in dementia (McCaddon and Hudson, 2010). Notably, both of these medical foods share some ingredients with the Perceptiv formulation and,

like the Perceptiv formulation, influence One-Carbon Metabolism (discussed in the following section.

Evidence exists for nutritional interventions for depression, including that which is observed in individuals diagnosed with AD (Varteresian and Lavretsky, 2014). The risk of depression is increased for older adults with low levels of vitamin B and high levels of homocysteine is high (Forti et al., 2010; Tiemeirer et al., 2002; Walker et al., 2010).

Several studies support an antidepressant effect of S-adenosyl methionine (SAM; Bottiglieri et al., 1990; Bressa, 1994). Individuals with depression have decreased levels of SAM and these levels increase in response to supplementation (Linnebank et al., 2010; Bottiglieri et al., 1990). SAM can augment pharmacological treatments for depression (Alpert et al., 2004; Papakostas et al., 2010). SAM has a beneficial effect on cognitive performance and mood for individuals diagnosed with AD and depression (Levkovitz et al., 2011), which may derive from enhancement of glutathione S-transferase activity, which is reduced in AD (Fontanari et al., 1994). This finding is consistent with the demonstration that SAM is an essential co-factor for this enzyme (Tchantchou et al., 2008).

Folate was effective in reducing depression (Papakostas et al., 2012). Individuals without dementia but with depressive symptoms but having normal levels of folate and B12 improved in cognitive performance (Walker et al., 2012).

Unlike current pharmacological agents, nutritional approaches have the potential to positively impact neurodegenerative processes well before manifestation of AD (Mi et al., 2013; Shea and Remington, 2012a, 2012b, 2015). *The studies reviewed in this petition demonstrate that dietary supplementation with the Perceptiv formulation meets this need. We will elaborate on these issues in the following sections.*

L. One-Carbon Metabolism impacts all aspects of Alzheimer neuropathology

One-Carbon Metabolism consists of two intersecting pathways: the Transmethylation Pathway and the Transulfuration Pathway (Fig. 1). In the Transmethylation Pathway, homocysteine is converted to methionine via Methylene Tetra-Hydrofolate Reductase (MTHFR) and methionine synthase with obligate cofactors folate and B12. Methionine is converted by methionine adenosyl transferase to S-adenosyl methionine (SAM). SAM mediates multiple essential methylation reactions by a number of SAM-dependent methyltransferases, yielding S-adenosyl homocysteine (SAH). SAH is reversibly converted to homocysteine by S-adenosyl Homocysteine Hydrolase (Bonetti et al., 2015); Dayon et al., 2017; Roman et al., 2019; Dalto and Matte, 2017).

Under conditions of insufficient methionine, or excess accumulation of homocysteine, homocysteine can alternatively combine with serine to form cystathione via cystathione beta synthase with vitamin B6 as an essential cofactor, then be converted to cysteine via cystathione- γ -lyase, then to glutamylcysteine via γ -glutamate cysteine synthase and ultimately to glutathione via glutathione synthase; this is known as the Transulfuration Pathway (Troesch et al., 2016; Sehlub, 1999; Vivitsky et al., 2006; McBean, 2012; Djurhuus et al. 1989; Roman et al., 2019; Finkelstein and Martin, 2000; Hensley and Denton, 2015). *The Transulfuration Pathway was originally considered to be absent from brain tissue but has more recently been demonstrated to be present and readily-manipulatable (Tallan et al., 1958; Vitvitsky et al., 2006).*

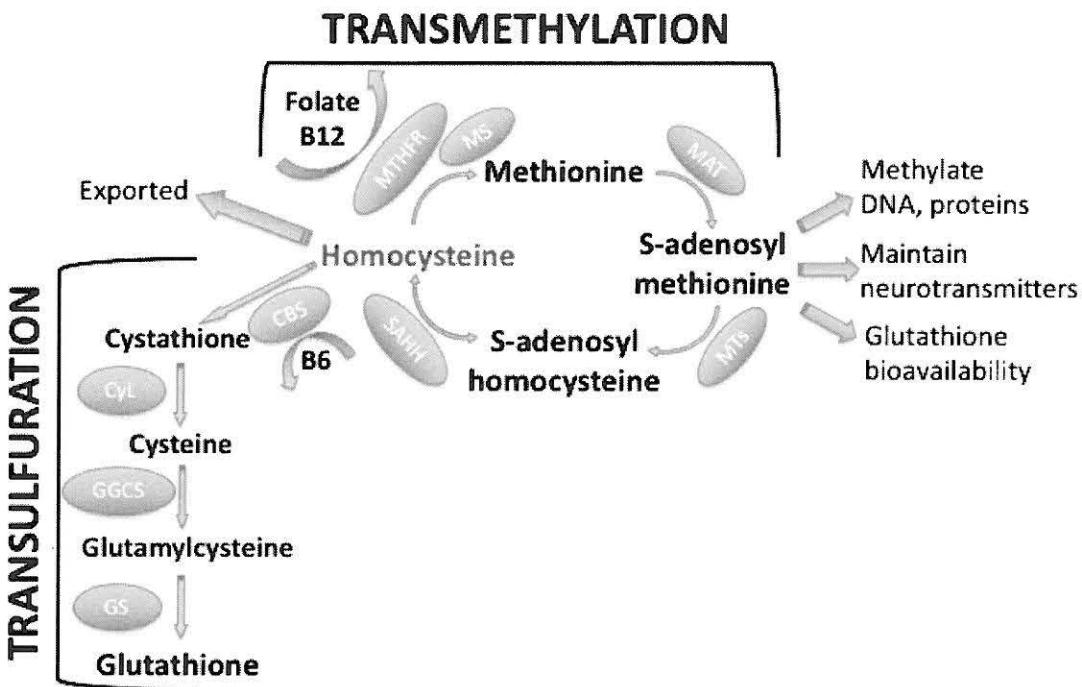


Fig. 1: One-Carbon Metabolism consists of the Transmethylation Pathway and the Transsulfuration Pathway. In the Transmethylation Pathway, homocysteine is converted to methionine via Methylene Tetra-Hydrofolate Reductase (MTHFR) and Methionine Synthase (MS) with obligate cofactors folate and B12. Methionine is converted by Methionine Adenosyl Transferase (MAT) to S-adenosyl methionine (SAM), which mediates multiple essential methylation reactions by a number of SAM-dependent methyltransferases (MTs), yielding S-adenosyl homocysteine (SAH). SAH is reversibly converted to homocysteine by S-adenosyl homocysteine hydrolase (SAHH). Alternatively, homocysteine can undergo cellular export or enter the Transsulfuration Pathway by conversion to cystathione by Cystathione Beta-Synthase (CBS) with obligate cofactor vitamin B6, followed by conversion to cysteine by Cystathione- γ -Lyase (Cyl), glutamylcysteine by γ -GlutamylCysteine Synthase (GGCS), and finally to glutathione via Glutathione Synthase (GS).

Both the Transmethylation and Transsulfuration Pathways reduce levels of homocysteine. However, the Transsulfuration pathway converts homocysteine to glutathione (therefore increasing antioxidant potential), while the Transmethylation Pathway converts homocysteine to methionine, which then generates SAM. One-Carbon Metabolism therefore mediates both methylation and antioxidant activity. Which of these pathways is favored depends upon levels of methionine. When methionine is abundant, relatively high levels of SAM are generated. Resultant high levels of SAM increase the activity of cystathione beta-synthase, which then shunts more homocysteine into the Transsulfuration Pathway, leading to increases in glutathione. By contrast, when methionine levels are low, SAM becomes limited and the Transsulfuration Pathway is therefore downregulated, which increases homocysteine entry into the Transmethylation Pathway (Prudova et al., 2006). Somewhat unexpectedly, dietary SAM supplementation did not significantly change plasma homocysteine levels in healthy adults (Thompson et al., 2009). Notably, this was determined by quantification of plasma rather than CSF levels, which do not necessarily reflect CSF levels (Thompson et al., 2009).

Deficient levels of the homocysteine re-methylation cofactors folate and B12 and of the transsulfuration cofactor B6 are commonly seen in the elderly population, fostering an increase in homocysteine with advancing age (Miller, 2003). Notably, dependence of both the

Transmethylation and Transulfuration Pathways on B vitamins to reduce homocysteine levels further underscores the impact of nutrition, and nutritional supplementation, in reducing the risk of AD (Taysi et al., 2015).

One-Carbon Metabolism impacts all aspects of AD neuropathology (Dayon et al., 2017) as follows:

•Multiple neurotoxic impacts of Homocysteine: Impairments in One-Carbon Metabolism result in increased levels of homocysteine. An international consensus statement (Smith et al., 2018) concluded that, based on the Bradford Hill criteria, an elevated level of total homocysteine is a recognizable risk factor for development of dementia and AD in older individuals. Individuals with high homocysteine levels had a >2-fold increase in the risk of onset of AD (Kivipelto et al., 2009). Homocysteine levels are elevated under conditions of folate and vitamin B12 deficiency (Bonetti et al., 2015); supplementation with B vitamins reduces homocysteine levels (Hainsworth et al., 2016). Plasma homocysteine levels were significantly higher in individuals diagnosed with AD versus non-demented controls (Villa et al., 2009). In addition, a greater increase in homocysteine levels was also observed for individuals diagnosed with AD versus controls following methionine loading, highlighting deficiencies in One-Carbon Metabolism in individuals with AD. B₁₂ levels were negatively correlated with homocysteine levels ($p<0.05$), and positively correlated with the extent of homocysteine increase following loading only for individuals diagnosed with AD and not the non-demented control groups (Villa et al., 2009). A 6-year longitudinal study with 2,570 individuals who did not have dementia at baseline demonstrated that individuals with higher levels of folate and B12 displayed a higher ratio of methionine vs. homocysteine, a decreased rate of loss of brain tissue, and a reduced risk of incident dementia and AD (Hooshmand et al., 2019). Homocysteine has multiple deleterious effects related to AD neuropathology (reviewed in Moretti and Caruso, 2019) as follows:

S-adenosyl Homocysteine Hydrolase degrades SAH to adenosine and homocysteine (Malanovic et al., 2008; Tehlivets et al., 2004). However, this reaction is reversible, allowing elevated levels of homocysteine to generate increased SAH. This inhibits upstream SAM-dependent methylation events since SAH is a potent inhibitor of SAM-dependent methyltransferases (Visram et al., 2018). Elevated homocysteine therefore fosters a global decrease in cellular methylation (Kennedy et al., 2004; Wang et al., 1997; Yi et al., 2000; Caudill et al., 2001; Selhub and Miller, 1992). This feedback inhibition further compromises the already reduced levels of SAM and diminished levels and activity of methyltransferases in brain tissue of individuals with AD (Kennedy et al., 2004; Morrison et al., 1996; Trolin et al., 1994).

Increases in SAH resulting from elevated homocysteine inhibit SAM-dependent methylation of DNA (Fuso, 2013). This decrease in DNA methylation increases presenilin expression, fostering increased gamma-secretase activity and resultant increased levels of Abeta (Fuso et al., 2012; Chan and Shea, 2007a; Chan et al., 2008b, 2009a). As seen above, this increases and potentiates intracellular reactive oxygen species, and SAH-mediated inhibition of methylation simultaneously inhibits glutathione-mediated quenching of these reactive oxygen species since SAM-dependent methylation is essential for glutathione S-transferase activity (Tchantchou et al., 2008). In addition to increasing Abeta levels, homocysteine potentiates Abeta-induced oxidative stress (Ho et al., 2001). Homocysteine further reduces glutathione efficacy by reducing expression of glutathione peroxidase, which is an additional essential enzyme for glutathione-mediated quenching of reactive oxygen species (Upchurch et al., 1997; Dalto and Matte, 2017).

Homocysteine induced calcium influx, glutamate excitotoxicity, kinase hyperactivation and apoptosis in cell culture models (Ho et al., 2001, 2002, 2003). Administration of homocysteine to rat pups promoted lipid and protein oxidation (Kooohpeyma et al., 2019). Increased homocysteine

levels resulted in increased N-homocysteinylation of tau and MAP1, which interferes with binding of each of these proteins to microtubules; this has been considered to represent one mechanism of brain aging (Bossenmeyer-Pourié et al., 2019).

•AD biomarkers: As described above, oxidative stress is an early event in AD neuropathology. Not only does perturbation of One-Carbon Metabolism induce oxidative damage (Tchantchou et al., 2005a), but resultant increased homocysteine augments Abeta-induced oxidative damage and fosters calcium influx by over-activation of NMDA channels (Ho et al., 2001; Tjiattas et al., 2004) and is associated with impaired cognition including that of individuals diagnosed with MCI (Kang et al., 2008; Sala et al., 2008; Moustafa et al., 2012). Homocysteine-mediated calcium influx fosters increased levels of phospho-tau by stimulation of kinase activity and simultaneous inhibition of corresponding phosphatase activity (Chan et al., 2008c). Accumulation of homocysteine is thought to induce tau hyperphosphorylation, leading to formation of neurofibrillary tangles, via a decrease in methylation (and therefore decreased activation) of Protein Phosphatase 2A, which dephosphorylates tau when activated (Vafai and Stock, 2002). Notably, methylation of Protein Phosphatase 2A is carried out by a SAM-dependent methyltransferase reaction (Lee and Stock, 1993).

A decrease in levels of glutathione represents an early event in neurodegeneration (Morozova et al., 2007), which contributes to oxidative damage induced by Abeta and potentiated by homocysteine (Ho et al., 2001). Activity of glutathione peroxidase (one of the enzymes that mediates quenching of oxidative species by glutathione) is essential for protection against oxidative damage to lipids in cells and in brain tissue from individuals diagnosed with AD (Yoo et al., 2010).

•Genetics related to AD: Compromises in One-Carbon Metabolism potentiate the deleterious impact of genetic risk factors for AD. Presence of one or more copies of the E4 allele of apolipoprotein (ApoE4) is the major genetic risk for AD (Corder et al., 1993). ApoE4 potentiates the deleterious influence of environmental factors, including nutritional deficiencies, on the risk of AD (Kivipelto et al., 2008; Lee et al., 2016). The negative influence of homocysteine, and the positive influence of B12, on cognitive function and brain cell survival was stronger in individuals diagnosed with AD that carried ApoE4 versus other ApoE alleles (Feng et al., 2009). ApoE4 also accentuated the deleterious influence of Abeta on cognitive function in cognitively normal individuals (Kantarci et al., 2012; Stomrud et al., 2010).

Mutations in genes encoding presenilin 1, tau and APP increase the risk of AD (Kelleher and Shen, 2017; Karch and Goate, 2015; Benitez et al., 2013; Poorkaj et al., 2001; Ringman et al., 2014). Supplementation with SAM suppressed the increase in Abeta, including its deposition as plaques, and suppressed the increase in phosphorylated tau in triple-transgenic mice expressing mutations in presenilin 1, APP and tau (Lee et al., 2012). Impaired methylation, resulting from inhibition of One-Carbon Metabolism either due to nutritional deficiencies or mutations in MTHFR, also fostered increased expression of genetically-normal presenilin 1, leading to overactivation of gamma-secretase and increased levels of Abeta (Fuso, 2013; Fuso et al., 2011a,b, 2012; Chan et al., 2008b, 2009a).

Just as mutations in MTHFR are associated with elevated homocysteine by reducing the efficacy of the Transmethylation pathway in One-Carbon Metabolism (Fuso, 2013; Fuso et al., 2011a, 2011b, 2012), mutations in Transulfuration enzymes are also associated with elevated homocysteine. Mutations in cystathionine beta-synthase (CBS) and cystathionine-γ-lyase (CγL) each foster elevated homocysteine and prevent downstream generation of glutathione (McBean, 2012; Roman et al., 2019; Pepe et al., 1999).

Detailed studies with cultures and mouse models demonstrate that perturbation in the Transmethylation Pathway increases Abeta and phospho-tau, and decreases neurotransmitter levels and cognitive performance, all of which are accentuated by homozygous ApoE knockout or expression of human ApoE4 (Chan et al., 2008b, 2009a, b; Shea et al., 2004). Perturbation of the Transmethylation Pathway by folate deficiency fostered compensatory increases in expression and activity of associated enzymes including MTHFR and cystathione beta synthase, with a corresponding decrease in expression and activity of methionine synthase, all of which are potentiated by ApoE deficiency in a gene-dosage manner (Tchantchou et al., 2006b). Perturbation of the Transmethylation Pathway also fosters a compensatory increase in transcription and activity of glutathione synthase under conditions of ApoE deficiency (Tchantchou et al., 2004). However, impaired generation of SAM resulting from perturbation of this pathway inhibits bioavailability of glutathione, since SAM is an obligate cofactor for glutathione S-transferase (Tchantchou et al., 2008). SAM has antioxidant effects in rat brain tissue that manifest as reduction of lipid peroxide production and as an enhancement of glutathione levels and activity (De La Cruz et al., 2000; Villalobos et al., 2000); Tchantchou et al., 2008).

•Cognitive performance: Elevated homocysteine is associated with a higher prevalence of cognitive and functional impairment and dementia even without deficiencies in B vitamins (Bonetti et al., 2015; Ellison et al., 2004). The impact of B vitamin supplementation on cognitive performance is related to basal levels of these vitamins. Reduced folate and elevated homocysteine concentrations in plasma are associated with poor cognitive performance in the general population in some but not all studies (Durga et al., 2007; Ellison et al., 2004). Low plasma B12 combined with high plasma folate reduced the risk of cognitive impairment versus moderate levels of each vitamin (Doets et al., 2014). B12 supplementation did not prevent cognitive decline in older diabetic patients with borderline B12 levels (Kwok et al., 2017).

Detailed studies with cultures and mouse models demonstrate that perturbation in the Transmethylation Pathway reduces choline and acetylcholine, fostering decreased cognitive performance and increased aggression in murine models (Chan et al., 2008b) and reduction of coordinated synaptic signaling in cortical cultures (Serra et al., 2008).

Low serum folate levels are strongly associated with atrophy of the cerebral cortex, and severity of atrophy is further directly correlated with progressively lower folate levels (Snowdon et al., 2000). In addition, while levels of folate in cerebral spinal fluid are normally three- to four-fold higher than in blood, spinal fluid levels of folate are significantly lower in AD patients (Serot et al., 2001; Wevers et al., 1994). Diminished folate levels are associated with a two-fold increased risk in developing AD, while normal cognitive scores were strongly associated with increased plasma levels of folate even in the presence of neuropathology (Wang et al., 2001). Notably, folate and B12 supplementation did not improve cognitive performance for those individuals with mild-moderate AD that were not previously deficient in these vitamins (Aisen et al., 2008).

•Acetylcholine and Synaptic Membranes: Choline metabolism intersects with One-Carbon Metabolism at the point where homocysteine is converted to methionine (Zeisel et al., 1989; Tchantchou, 2006; Fig. 2). Choline levels are therefore critically dependent upon One-Carbon Metabolism. Acetylcholine levels are dependent upon choline (reviewed in Tchantchou and Shea, 2008). Similarly, levels of phosphatidyl choline, required for membranes, (including synaptic membranes), are dependent upon choline (Li and Vance, 2008; Obeid and Herrmann, 2009), which declines during AD as a possible consequence of cholinesterase inhibitor therapy (Wurtman, 2015). Moreover, since choline can be utilized as an alternative methyl donor, chronic impairments in One-Carbon Metabolism could foster reduction in both acetylcholine and

phosphatidylcholine due to shunting choline through the Transmethylation Pathway (Tchantchou and Shea, 2008). Furthermore, levels of SAM levels and the activity of associated methyltransferases are reduced in brain tissue of individuals with AD (Kennedy et al., 2004; Selhub and Miller, 1992; Obeid and Herrmann, 2009), which would be expected to contribute to acetylcholine deficiency by the above interactions.

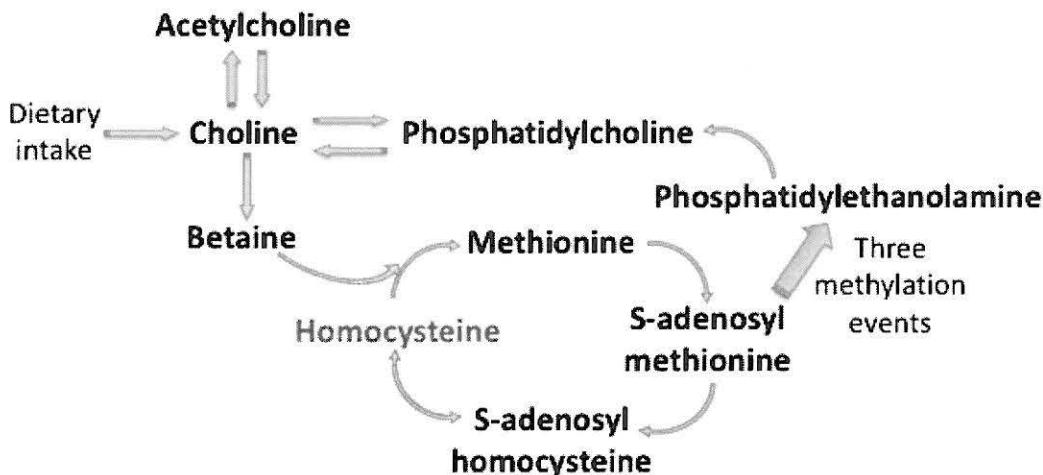


Fig. 2: Interaction of Choline and One-Carbon Metabolism. In the absence of sufficient dietary intake of choline, choline is generated by SAM-mediated methylation of phosphatidylethanolamine three successive times, followed by hydrolysis to phosphatidylcholine (which can be used for membranes) or reversible dephosphorylation to yield choline. Resultant choline can be acetylated to generate the neurotransmitter acetylcholine. In the absence of sufficient SAM, choline can serve as an alternative methyl donor and be utilized to convert homocysteine to methionine, then to SAM, which can then methylate multiple intracellular targets as described above. Notably, divergence of choline into the Transmethylation Pathway can compromise neurotransmitter levels as well as membrane biogenesis and maintenance.

Evidence that any single nutrient has a beneficial effect on cognition or lowers the risk for AD is limited, but this is to be expected given the multiple neuronal compromises and multiple neuropathologies observed during aging and AD (Engelborghs et al., 2014). Choline, however, may be unique in that it is the direct precursor to the neurotransmitter acetylcholine, which undergoes progressive, critical decline in AD (Hampel et al., 2018). Fostering increased production of acetylcholine by consumption of a single metabolite is a preferred approach to attempting to preserve continuously declining levels via cholinesterase inhibition. In addition, increased dietary choline and/or SAM represent potential beneficial approaches in combination with cholinesterase inhibition (Shea, 2019; Tchantchou et al., 2006a).

As described above, choline is the precursor of acetylcholine and directly influences acetylcholine synthesis in brain tissue (Cohen and Wurtman, 1975). Choline is also the precursor for phosphatidylcholine, which is required for membrane synthesis and maintenance of synaptic function (Li and Vance, 2008; Wurtzman, 2015). Meta-analysis demonstrates that individuals with AD have reduced choline in both plasma and cerebrospinal fluid (De Wilde et al., 2017). While choline concentrations are higher in brain than in plasma, brain levels are critically dependent upon the circulatory system and depend on plasma concentrations because of the kinetic characteristics of the blood-brain-barrier transport system. When cholinergic neurons are activated, acetylcholine release can be enhanced by treatments that increase plasma choline (for example, consumption of certain foods; Blusztajn and Wurtman, 1983; Ylilaruri et al., 2019). Vegetarian-based diets harbor the potential to provide insufficient dietary choline (Derbyshire, 2019) and may require supplementation.

In addition to preserving synaptic membranes and neurotransmitter levels (both of which undergo critical decline in AD), choline metabolism intersects with One-Carbon Metabolism at the point where homocysteine is converted to methionine (Zeisel et al., 1989; Bonetti et al., 2015). Choline levels are therefore critically dependent upon folate and B12 (reviewed in Tchantchou and Shea, 2008), since both are essential for conversion of homocysteine to methionine. Neurons can synthesize choline by SAM-dependent methylation of phosphatidylethanolamine followed by hydrolysis to phosphatidylcholine. Since SAH inhibits SAM-dependent methyltransferases, it inhibits phosphatidylcholine synthesis (which requires three SAM-dependent methylation events. (Malanovic et al., 2008; Tehlivets et al., 2004). All methyl groups of choline are in fact derived by SAM-dependent transmethylation (Bremer et al., 1960). Accordingly, methionine (following conversion to S-adenosylmethionine) regulates choline levels (Zeisel et al., 1989). This was demonstrated by restoration of choline and acetylcholine levels following SAM supplementation in aged mice and in mice expressing human ApoE4 and in mice harboring a mutation in MTHFR that limits folate-mediated conversion of homocysteine to methionine (Chan et al., 2008b). Folate deficiency also reduced, while SAM supplementation restored, N-methyl nicotinamide in brain tissue, which inhibits choline transport out of the central nervous system (Williams and Ramsden, 2005). Since choline can be utilized as an alternative methyl donor, chronic impairments in One-Carbon Metabolism could foster acetylcholine reduction. In this regard, levels of SAM levels and activity of associated methyltransferases are reduced in brain tissue of individuals with AD (Kennedy et al., 2004; Selhub and Miller, 1992), which would be expected to contribute to acetylcholine deficiency by the above interactions.

•Epigenetics: One-Carbon Metabolism plays an ongoing epigenetic role by modulation of DNA methylation, including memory formation (Fischer, 2014; Fuso et al., 2011a, 2011b, 2012; Fuso, 2013). Adequate levels of SAM, the major methyl donor, are required for DNA methylation (Shea and Remington, 2015; Tchantchou et al., 2006a). Deficiencies in methylation can be reversible, highlighting the potential for nutritional intervention that stimulates One-Carbon Metabolism as part of a therapeutic approach (Dauncey, 2014; Fuso and Scarpa, 2011; Shea and Remington, 2015; Tchantchou and Shea, 2008; Shea and Chan, 2008). Methylation events mediated by this cycle play a critical role in neuronal homeostasis throughout life, and impaired function of this cycle is associated with AD (Fuso et al., 2005; Montgomery et al., 2014; Lunnon et al., 2014; Shea and Rogers, 2014; Nicolia et al., 2014; Sanchez-Mut et al., 2013; Roubroeks et al., 2017; Ciceri et al., 2017). DNA methylation is reduced in brain tissue of individuals with AD, due to reduced expression of methyltransferases and deficiency in vitamins B2, B6, B12, and folate (Wang et al., 2013; Lardenoije et al., 2015; Landgrave-Gomez et al., 2015; Mastroeni et al., 2011; Lu et al., 2013; Teijido and Cacabelos, 2018; Roubroeks et al., 2017; Coppede et al., 2012). Most if not all of the genes known to increase the risk of AD are subject to hypomethylation, and in some cases, undergo hypomethylation to an extent beyond that of global hypomethylation. PSEN-1, the gene that codes for presenilin, is particularly susceptible to overexpression following hypomethylation, which sets off the cascade leading to beta- and gamma-secretase overactivation and generation of increased Abeta; preclinical studies demonstrate that SAM prevents this cascade, including under conditions of folate and B12 deprivation (Montgomery et al., 2014; Shea and Chan, 2008; Fuso, 2013; Fuso et al., 2005, 2011a,b, 2012; Nicolia et al., 2014; Do Carmo et al., 2016; Yan and Vassar, 2014). The analogous promoter was observed to be hypomethylated in postmortem examination of brain tissue of individuals diagnosed with AD (Do Carmo et al., 2016). Beta secretase 1 itself is also overexpressed in brain tissue of individuals diagnosed with AD due to hypomethylation under conditions of folate and B12 deficiency, which will increase Abeta levels (Marques et al., 2012). The APP promoter is also highly susceptible to overexpression under conditions of hypomethylation (Iraola-Guzmán et al., 2011). Deficiency in B vitamins in individuals diagnosed with AD reduces methylation of the

glycogen synthase kinase 3 β promoter, which increases expression of this kinase; notably this kinase induces tau phosphorylation, fostering neurofibrillary tangle formation, leading to cytoskeleton dysregulation and cell death (Nicolia et al., 2010). The unique sequence of the APOE4 promoter is considered uniquely susceptible to hypomethylation, which can increase APOE4 expression in individuals diagnosed with AD (Wang et al., 2008, 2013; Caeser and Grandy, 2012). Accordingly, both the presence of key mutations, coupled with overexpression due to impaired promoter methylation, can increase the risk of AD.

Epigenetic drift is observed in association with aging in individuals diagnosed with AD and may contribute to differential risk and onset of AD (Wang et al., 2008; Irier and Jin, 2012). Epigenetic drift can be caused by multiple factors, including lifestyle, diet, folate status and homocysteine status (Bestor et al., 2015). Environmental events foster epigenetic modifications, including DNA methylation. Epigenetics may therefore be one link between environmental and genetic risk factors for AD (Iraola-Guzman et al., 2011; Roman et al., 2019).

M. The Perceptiv formulation supports One-Carbon Metabolism, increases levels and bioavailability of the endogenous antioxidant glutathione, provides additional antioxidant protection to membranes and supplies cellular energy.

The Perceptiv formulation supports and enhances multiple aspects of One-Carbon Metabolism, including both the Transmethylation and Transsulfuration Pathways, provides antioxidant protection to cell membranes and synapses, maintains ATP levels and provides protection to mitochondria (Fig. 3).

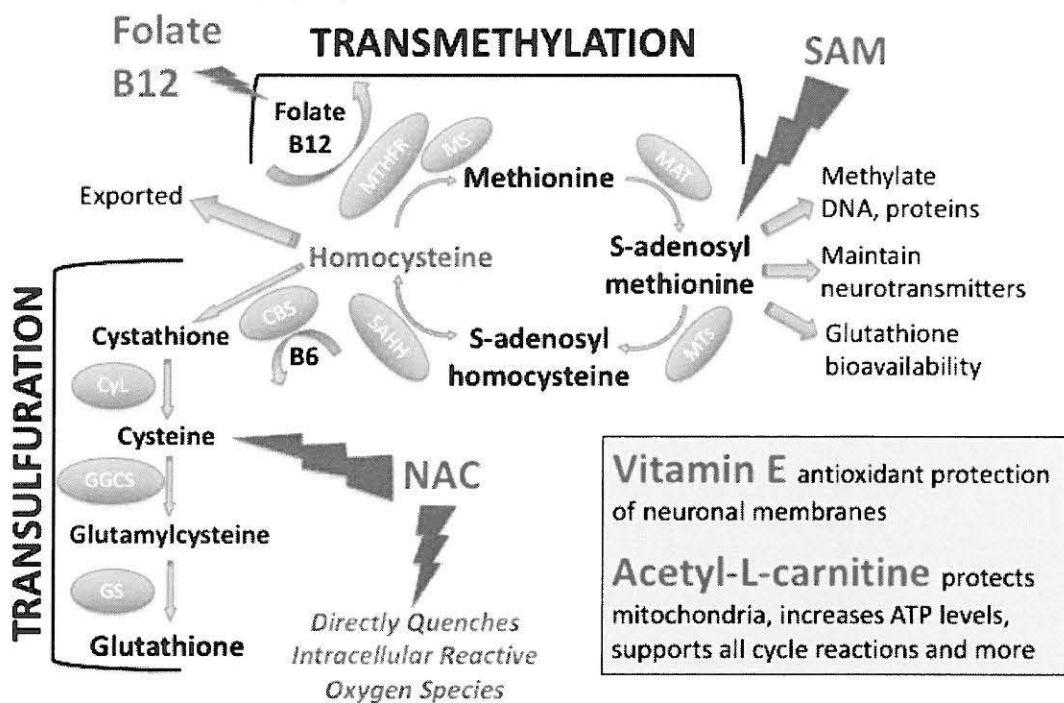


Fig. 3: One-Carbon Metabolism is boosted at multiple points by the Perceptiv formulation (Folate, B12, SAM, NAC, Vitamin E and ALCAR). Folate and B12 are essential cofactors for conversion of homocysteine to methionine, which both reduces homocysteine and boosts the Transmethylation Pathway. SAM provides additional methylation potential to protect DNA and proteins, maintain acetylcholine, and increase glutathione bioavailability. N-acetyl cysteine (NAC) both directly quenches intracellular reactive oxygen species and provides cysteine to support the Transmethylation Pathway to restore levels of the antioxidant glutathione, which also quenches intracellular reactive oxygen species using SAM as an

essential cofactor. Vitamin E provides protection against oxidative damage to membranes, which would otherwise foster additional internal oxidative damage and inflammation and consume glutathione. Acetyl-L-carnitine (ALCAR) protects mitochondria and maintains/increases ATP levels, providing energy for these as well as other neuronal reactions. See text for references.

The Perceptiv formulation (Folate, B12, SAM, NAC, Vitamin E and ALCAR) supports the Transmethylation Pathway, using folate and B12 to stimulate MTHFR- and methionine synthase-mediated conversion of homocysteine to methionine and eventually to SAM, maintaining overall cellular methylation. Deficiencies in the homocysteine re-methylation cofactors folate and B12, and in the Transsulfuration Pathway cofactor B6, are commonly seen in the elderly, fostering an increase in homocysteine with advancing age (Miller, 2003). Folate, B12 and SAM are components of One-Carbon Metabolism: methylation events mediated by this cycle play a critical role in neuronal homeostasis throughout life, and impaired function of this cycle is associated with AD (Fuso et al., 2005). As described above, mutations in MTHFR that reduce this enzyme's activity foster a requirement for additional folate and SAM (Roman et al., 2019). Genetic deficiencies in enzymes of the Transsulfuration Pathway as described above shifts the full burden of homocysteine elimination to the Transmethylation Pathway, resulting in a greater burden on MTHFR and cofactors folate and B12. This highlights a critical need for supplementation with folate and B12 for individuals harboring mutations in cystathione beta-synthase or cystathione- γ -lyase as well as mutations in MTHFR. It is important to supplement with both folate and B12, since they work in tandem to support the Transmethylation Pathway: supplementation with folate can mask and potentially exacerbate B12 deficiency (e.g., see Moretti and Caruso, 2019).

Clinical studies demonstrate that the Perceptiv formulation and its components improve and maintain cognitive performance for individuals with no known or suspected cognitive difficulties, for individuals diagnosed with MCI, and for individuals diagnosed with AD. Clinical and preclinical studies demonstrate that components of the Perceptiv formulation support aspects of neuronal health directly related to AD including reducing oxidative damage, reducing homocysteine levels, increasing glutathione levels and bioavailability, increasing acetylcholine levels, increasing cognitive performance, reducing aggression, increasing DNA methylation, reducing presenilin-1 expression, reducing secretase activity, reducing intracellular and extracellular Abeta deposits, and reducing phospho-tau levels. *These studies are reviewed and referenced in Section III.*

As described above, levels of the neurotransmitter acetylcholine, which decline progressively in AD (Hampel et al., 2018) are critically dependent upon One-Carbon Metabolism. Accordingly, these findings warrant nutritional intervention to support One-Carbon Metabolism and associated levels of folate, B12 and SAM. Supplementation with folate, B12 and SAM represent one potential strategy to maintain choline, and therefore acetylcholine levels, which can reduce the risk of AD.

Maintenance of glutathione levels and bioavailability, which result from supplementation with folate and B12, SAM and/or NAC, can offset the decreases in these components observed in AD and, in doing so, quench Abeta- and homocysteine-mediated oxidative damage (Morozova et al., 2007; Yoo et al., 2010).

Along these lines, we found the maximum impact of a combinatorial formulation (subsequently termed Perceptiv) that targeted One-Carbon Metabolism and provided an energy source for mitochondrial function (folate, vitamin B12, vitamin E as alpha-tocopherol, S-adenosyl methionine, acetyl-L-carnitine and N-acetyl cysteine) on adults (including elderly) with no known or suspected cognitive difficulties, followed by individuals diagnosed with MCI, and followed in succession by individuals with mild, moderate and severe AD (Chan et al., 2008a, 2010;

Remington et al., 2015a,b, 2016; Shea and Remington, 2019; these are presented in detail below in Section III).

The Perceptiv formulation was derived from an extensive series of laboratory investigations with neuronal cultures and transgenic and/or aged mice spanning >20 years. While such studies are by definition far afield of clinical trials, they nevertheless afford the unique ability to examine models of at-risk populations while they are still apparently healthy and cognitively intact, as well as to initiate treatments prior to symptomatic onset (Shea and Remington, 2012a). The degree of control and manipulation provided by such studies affords considerable insight into underlying mechanisms to an extent virtually impossible to achieve during clinical trials and that cannot be readily examined in humans. As such, essential data is provided by model systems. As the following studies demonstrate, the ingredients of the Perceptiv formulation delay or prevent age-related neurodegeneration, via multiple approaches directly relevant to the human condition:

Folate protects against neuronal inflammation and degeneration, reduces homocysteine and oxidative damage, reduces production and toxicity of Abeta, and maintains appropriate neuronal calcium levels (Kifle et al., 2009; Tjiattas et al., 2004; Zhang et al., 2008; Dhitavat et al., 2005; Li et al., 2015; Liu et al., 2016). Folate maintains levels of S-adenosyl methionine (Schatz et al., 1981; Bottiglieri et al., 1990; Muller et al., 2001; Ho et al., 2003; Hyland et al., 1988) and, in doing so, restores DNA methylation including that impaired by Abeta accumulation (Li et al., 2015). Folic acid supplementation increased activity of methionine synthase and cystathione-beta synthase enzymes (indicating stimulation of both Transmethylation and Transulfuration pathways), increased glutathione and reduced homocysteine-induced protein oxidation in rats (Koopeyma et al., 2019).

B12 reduces homocysteine, regulates neuronal calcium levels and prevents apoptosis (Kifle et al., 2009). B12 prevents cognitive impairment in mice (Troen et al., 2008).

SAM is part of the formulation in order to maximize methylation potential and, in doing so, provide further protection to DNA and proteins. By maintaining methylation capacity, SAM reduces expression of presenilin-1, activity of gamma- and beta-secretases, and reduces Abeta production and formation of senile plaques (Chan and Shea, 2007a; Chan et al., 2009a; Lee et al., 2012; Fuso et al., 2005; Scarpa et al., 2003). Hypomethylation of the beta-secretase promoter fosters increased generation of Abeta in transgenic mice, which was alleviated by SAM supplementation; notably, the analogous promoter was observed to be hypomethylated in postmortem examination of brain tissue of individuals diagnosed with AD (Do Carmo et al., 2016). SAM reduces levels of phosphorylated tau (Chan and Shea, 2006a; Chan et al., 2008c, 2009b; Lee et al., 2012). SAM regulates activity of neuronal membrane channels and phosphatase activity (Chan et al., 2008c). SAM reduces oxidative stress (De La Cruz et al., 2000, 2002; Villalobos et al., 2000; Tchantchou et al., 2006a, 2008) and regulates levels of glutathione (Villalobos et al., 2000) as well as the activity of glutathione S-transferase, which increases glutathione-mediated antioxidant potential (Shea and Chan, 2008; Tchantchou et al., 2008). SAM maintains levels of choline and the neurotransmitter acetylcholine (Chan et al., 2008b), stimulates synaptic activity (Serra et al., 2008) and improves cognitive performance and reduces aggressive behavior in mice (Chan et al., 2008b; Sontag et al., 2007). SAM also maintains neuronal health by supporting myelination (Surtees et al., 1991).

N-acetyl Cysteine (NAC) is a precursor of L-cysteine and, in turn, a precursor of glutathione (Tardiolo et al., 2018; Arakawa, 2007). NAC enters the Transulfuration Pathway via conversion to cysteine and increases glutathione levels, which increases antioxidant potential (Aldini et al., 2013; Huang et al., 2010; Tchantchou et al., 2005a). Direct intake of glutathione is insufficient to

replenish required levels, especially with respect to brain tissue since it is rapidly hydrolyzed and has a limited capacity to cross the blood-brain barrier (Witschi et al., 1992). Similarly, L-cysteine intake fails to replenish glutathione due to metabolic limitations (Vina et al., 1978; Sjodin et al., 1989; Borghstrom and Kagedal, 1990). By contrast, oral consumption of NAC increases systemic L-cysteine and, subsequently, glutathione and, since NAC readily crosses the blood-brain barrier, it also increases glutathione in brain tissue (Holdiness, 1991; Lavoie et al., 2008; Neuwelt et al., 2001; Farr et al., 2003). NAC can suppress the apoptotic enzyme cascade and therefore may provide additional neuroprotection and reduce ultimate neuronal loss that accompanies AD including that resulting from Abeta accumulation (Hsiao et al., 2008; Xu et al., 2009; Yan et al., 1998). Increased glutathione antioxidant capacity following supplementation with NAC alleviated aberrant calcium channel activation which otherwise potentiated Abeta-induced impairments in spatial memory in rats (More et al., 2018). As such, NAC has been tested as an alternative to glutathione for treatment of dementia with some success (reviewed in Hara et al., 2017).

In addition to replenishing glutathione, NAC harbors its own antioxidant as well as anti-inflammatory properties, both of which provide neuroprotection (Omara et al., 1997; Tardiolo et al., 2018; Arakawa, 2007; Fuller et al., 2010; Lee et al., 2010a,b; Pahan et al., 1998; Farr et al., 2003; Paintlia et al., 2008; Unnithan et al., 2013). NAC provides oxidative protection to membranes including synaptosomes, augmenting that of glutathione itself, against damage caused by Abeta and 4-hydroxy-nonenol, the latter of which is generated by lipid peroxidation (Koppal et al., 1999; Pocernich et al., 2001). NAC furthermore prevents downstream oxidation of proteins by both Abeta, 4-hydroxy-nonenol and acroein (Fu et al., 2006; Huang et al., 2010) and as such complements external antioxidant protection provided by vitamin E (which is restricted to protection of cell membranes (Dhitavat et al., 2001). Decreased neuronal antioxidative capacity accompanies aging of triple transgenic 3xTg-AD mice, which was alleviated by extracellular cysteine (Dong et al., 2019). NAC supports cognitive performance (Farr et al., 2003) and prevented cognitive decline and apoptosis otherwise resulting from Abeta (Fu et al., 2006; Xu et al., 2009). NAC increases the activity of Glutathione Peroxidase and Glutathione-Disulfide Reductase (which mediate reduction of oxidative species by glutathione; Huang et al., 2010).

Vitamin E does not directly enter One-Carbon Metabolism, but rather provides antioxidant protection to neuronal membranes (Butterfield et al., 1999; Cobb and Cole, 2015; Subramaniam et al., 1998). In doing so, it reduces the requirement for membrane repair which therefore lessens consumption of SAM to generate phosphatidylcholine. By providing antioxidant protection to membranes, Vitamin E furthermore reduces radiation of reactive oxygen species derived from membranes to internal components, which therefore lessens consumption of NAC and glutathione (as well as the glutathione cofactor SAM). Vitamin E also supports cognitive performance (Nishida et al., 2006; Ulatowski and Manor, 2015). Interestingly, combined administration of the pharmaceutical memantine along with vitamin E did not provide any additional benefit over supplementation with vitamin E alone (Dysken et al., 2014).

Acetyl-L-Carnitine (ALCAR) also does not directly enter One-Carbon Metabolism, but rather protects mitochondria, and in doing so, maintains/increases ATP levels, which provides energy to maximize One-Carbon Metabolism as well as overall neuronal reactions (Dhitavat et al., 2001, 2002). ALCAR supports production of glutathione (Dhitavat et al., 2002; Aureli et al., 1999). ALCAR benefits cognition and memory in rats including during aging (Barnes et al., 1990; Markowska and Olton, 1990) and in humans diagnosed with MCI and AD (Stuart et al., 2003).

Combinations of multiple ingredients of the Perceptiv formulation highlight additional benefits to neuronal homeostasis and mitigation of AD-related pathological events:

Folate plus B12 provide superior attenuation of oxidative damage, prevention of apoptosis, reduction of homocysteine, and regulation of calcium levels than either vitamin alone (Kifle et al., 2009). Folate plus B12 increase levels of S-adenosyl methionine (Fuso et al., 2008), and DNA methylation, therefore regulating overall gene expression and in particular expression of presenilin-1 (Fuso et al., 2011a, b).

Folate plus vitamin E attenuate genetically-induced oxidative damage to brain tissue (Shea and Rogers, 2002), reduce tau hyperphosphorylation, (Chan and Shea, 2006a), regulate expression of genes of the methionine cycle (Tchantchou et al., 2006b), and improve cognitive performance in mice (Milhalik et al., 2004).

Folate plus vitamin E plus ALCAR provides synergistic neuroprotection against Abeta-induced oxidative damage that exceeds any one of these or combination of any two (Dhitavat et al., 2005).

S-adenosyl methionine plus NAC and ALCAR regulate aggressive behavior in mice (Chan and Shea, 2007b)

The results of the above laboratory investigations are summarized in Table 1.

Table 1: Ingredients of the Perceptiv Formulation Have a Positive Impact on Neuronal Biomarkers Related to General Health as well as to MCI and AD, and in Doing so Reduce the Risk of AD

Ingredient(s) of the Formulation	Effect on Brain Tissue
Folate, B12, Vitamin E, NAC, SAM	Reduction of Oxidative Damage
Folate, B12, NAC, SAM	Reduction of Homocysteine Levels and Toxicity
Folate, B12, NAC, ALCAR, SAM	Increased Levels and Bioavailability of Glutathione
Folate, SAM	Support of Synaptic Activity and Complex Signaling
ALCAR	Support of Mitochondrial Health and Activity
Folate, B12, SAM	Reduction of Presenilin-1 Expression
Folate, B12, SAM	Reduction of Beta and Gamma-Secretase Activity
Folate, B12, SAM	Reduction of Beta-amyloid Levels
Folate, B12, SAM	Reduction of Phosphorylated Tau Levels
Folate, B12, SAM	Maintenance of Acetylcholine Levels

Since the components of the Perceptiv formulation address multiple ongoing neuropathological events, the formulation can maximize impact for individuals at various stages of neuronal challenge and cognitive compromise. For example, vitamin E may be the most important factor during the early period of Abeta overexpression (when membranes, including synaptic membranes, undergo oxidative challenge), while N-acetyl cysteine may be crucial at later stages, when internal oxidative damage is rampant. Folate and B12 may be critical for elimination of homocysteine and generation of SAM, but if methionine levels are adequate (which would foster generation of ample SAM regardless of folate or B12), elimination of homocysteine would rely heavily on the Transulfuration Pathway, where N-acetyl cysteine would be critical for homocysteine elimination, along with resultant increased levels of glutathione. At all times, and under all conditions, ALCAR maintained/increased generation of ATP.

Stimulation of One-Carbon Metabolism by supplementation with folate and vitamin E can also mitigate a genetic risk for AD. The presence of ApoE4 can augment deficiency in One-Carbon Metabolism (Fluegge, 2019a, b). Underlying mechanisms were previously probed in a

mouse model. When adult transgenic mice lacking murine apolipoprotein E (ApoE^{-/-}) and expressing the human E4 isoform (a genetic risk factor for AD and which increases oxidative damage to brain tissue; Kamboh, 2004) were maintained on a diet lacking folate and vitamin E, they displayed significantly increased oxidative damage to brain tissue and significantly decreased cognitive performance in maze trials. This deficient diet did not induce oxidative damage or cognitive decline in normal mice (Shea and Rogers, 2002; Chan et al., 2008b). By contrast, these transgenic mice demonstrated no deleterious effects provided they were maintained on a complete diet (i.e., supplemented with folate and Vitamin E). One interpretation of these findings is that supplementation buffered the deleterious impact of a known genetic predisposition that would otherwise impair cognitive performance. Extension of these findings to the human condition is consistent with the notion that genetic predispositions may remain latent pending age-related decline in nutrition and/or homeostasis (Bayer et al., 2006; Mattson and Shea, 2003). A similar effect was observed with normal mice aged 2.5–3 years: normal aged mice performed equally well in maze trials as did 1-year old mice when maintained on a complete diet, but displayed statistically reduced cognitive performance when maintained on the above deficient diet (Chan et al., 2008b). The beneficial effects of the complete diet in these studies were likely to be derived from mobilizing endogenous antioxidants as well as by the direct antioxidant effect of dietary vitamin E (Tchantchou et al., 2008; Shea and Remington, 2012a, 2012b).

As stated above, an increase in homocysteine levels is a recognizable risk factor for development of dementia and AD in older individuals (Smith et al., 2018). *This strongly supports the Perceptiv formulation, components of which reduce homocysteine levels and mitigate multiple pathological consequences of elevated homocysteine, as reducing the risk of AD.*

N. Statement of Relevance

This petition addresses all elements set forth in the FDA document, “Guidance for Industry: FDA’s Implementation of Qualified Health Claims”², linked documents found within this parent document, and those from additional relevant FDA documents and references to same (e.g., Rowlands and Hoadley, 2006; Ellwood et al., 2010) as noted throughout this petition.

II. PRELIMINARY REQUIREMENTS

A. Description of the Supplement

The commercial product Perceptiv[®] is comprised of the identical formulation in all respects that was utilized in all of the clinical studies reviewed herein (Chan et al., 2008a, 2010; Remington et al., 2009, 2015a, b, 2016; Shea and Remington, 2018a, 2018b, 2019). In these studies, which were completed prior to establishment of the commercial product, this formulation was referred to by the generic term “Nutraceutical Formulation” (abbreviated in these studies as “NF”). For simplicity, we shall refer to the formulation by its commercial name, Perceptiv; since future commercial ventures could result in a change in product name, and we therefore stress the “Perceptiv formulation” to specify that Qualified Health Claims are sought for the formulation regardless of any change in product name. Recently, we carried out important retrospective analyses of our clinical data, resulting in additional publications. The timeline of clinical studies and marketing is presented in Table 2.

²<https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-evidence-based-review-system-scientific-evaluation-health-claims>. Last accessed 10/19/2019.

Table 2: Timeline of Clinical Studies and Marketing of the Perceptiv formulation

Time Line	Event(s)	Publications
2006-2008	Phase I studies with the "Nutraceutical Formulation"	Chan et al., 2008a Remington et al., 2009 Chan et al., 2010
2009	Marketed as MemoryXL* Marketed as "GreatMind"	
2009-2012	Phase II studies with the "Nutraceutical Formulation"	Remington et al., 2015a Remington et al., 2015b Remington et al., 2016
2012-present	Marketed as Perceptiv**	
2018-2019	Retrospective examination of data of Chan et al., 2010 and Remington et al., 2015a	Shea and Remington, 2018a Shea and Remington, 2018b Shea and Remington, 2019

*Since the formulation was developed at UMass Lowell (Lowell, MA 01854), UMass Lowell is the owner. UMass Lowell licensed the formulation to a start-up company (USI) that marketed the formulation as "MemoryXL." This license was terminated within the same year due to contractual issues. The formulation was simultaneously marked briefly by Pharmavite as "GreatMind."

**Following completion of Phase II studies, and while these studies were in the process of publication, UMass Lowell licensed the formulation to Sevo Nutraceuticals, who markets the formulation as Perceptiv®.

The Perceptiv formulation is a dietary supplement consisting of 3 vitamins (Folic acid, B-12 and vitamin E as alpha-tocopherol) and 3 nutraceuticals (S-adenosyl methionine, N-acetyl cysteine and acetyl-L-carnitine) all of which are "Generally Recognized as Safe" (GRAS). The vitamins are included at their FDA-approved respective Recommended Daily Allowances³ as follows:

- Folic acid: 400µg
- B12: 6 µg
- Vitamin E: 30 I.U.

Recommended Daily Allowances have not been established for the 3 nutraceuticals. They were therefore included at concentrations commonly and historically available as individual over-the-counter supplements from a variety of manufacturers:

- N-acetyl cysteine: 600mg (e.g., Swanson Premium, Jarrow Formulas, Puritan's Pride, GNC)
- Acetyl-L-carnitine: 500mg (e.g., Swanson Premium, Jarrow Formulas, Puritan's Pride, GNC)
- S-adenosyl Methionine: 400mg (200mg of which is active ion; e.g., Jarrow Formulas, NatureMade)

B. The Perceptiv Fomulation Conforms to the Requirements of 21 C.F.R. § 101.14(b)

The Perceptiv formulation conforms to the requirements of 21 C.F.R. §101.14(b)⁴ as follows: the formulation provides essential nutrients, deficiency of which have been related to impaired neuronal function. As demonstrated within this petition, consumption of the Perceptiv formulation benefits cognitive performance for individuals across the adult life span with no known nor suspected cognitive difficulties, for individuals diagnosed with MCI and for individuals diagnosed

³<https://www.fda.gov/food/guidance-documents-regulatory-information-topic-food-and-dietary-supplements/labeling-nutrition-guidance-documents-regulatory-information>. Last accessed 10/19/2019.

⁴ www.ecfr.gov (Title 21, Chapter I, Subchapter B, Part 101, Subpart A, §101.14; last accessed 10/19/2019)

with multiple stages of AD.

C. The FDA Previously Recognized the Perceptiv Formulation as GRAS.

Multiple sites participated in the phase II studies of the Perceptiv formulation (these studies are described in detail below). On February 19, 2009, one of the participating sites submitted an Investigational New Drug (IND) Application for the use this formulation (then appearing briefly under the brand name "MemoryXL"; Table 1, above). The FDA at that time assigned the IND 104,721 and notified that participating site that investigation for potential deficiencies would be completed by March 22, 2009. No deficiencies were reported. A copy of this letter from the FDA is included in Appendix B.

D. Perceptiv Components are Associated with a Disease Affecting the General U.S. Population: Substance-Disease Relationship

Rowlands and Hoadley (2006) and Ellwood et al. (2011) provide a review of preparation and justification of Qualified Health Claims with regard to food and dietary supplements (FDA, 2018⁵). As described by Rowlands and Hoadley (2006) and in the referred FDA document (FDA, 2018), a health claim in the context of FDA approval suggests that a relationship exists between a substance in the food and a disease. Rowland and Hoadley (2006) further specify that, according to the FDA Guidance for Industry (FDA, 2018), there are therefore three components to a health claim: the substance, the disease, and the relationship linking the substance and disease. We will define these one by one:

A substance includes nutrients or other components of a food or a specific food. We reference herein studies on multiple foods, vitamins and supplements that impact cognitive performance, but for the purpose of this document, the substance is the Perceptiv formulation. Inherent to specification of the Perceptiv formulation as the substance is that supplements are recognized as food by the FDA (FDA, 2018).

Disease is defined as damage to an organ, structure, or system of the body such that it does not function; herein the disease is AD, the most common form of dementia (Alzheimer's Association Report, 2016a), in which the brain is the organ that is damaged leading to progressive loss of function.

The health-related condition is defined as a state of health leading to the above damage and loss of function. The nature of the substance/disease relationship addressed in authorized health claims is that of reduction of risk of the specified disease. Rowlands and Hoadley (2006) relate the FDA requirement that "the disease must be one for which the general U.S. population, or an identified population subgroup (e.g., the elderly), is at risk." In the context of this petition, the elderly is the identified population subgroup; however, since all individuals will eventually be elderly, and AD occurs world-wide with no known excluded racial or ethnic groups (Alzheimer's Association Report, 2016), the general U.S. population is ultimately at risk for AD (defined above as the disease). We elaborate on this point in Section VIII E (Who Will Benefit by the Addition of the Proposed Claims).

⁵ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-evidence-based-review-system-scientific-evaluation-health-claims>. Last accessed 10/19/19/2019

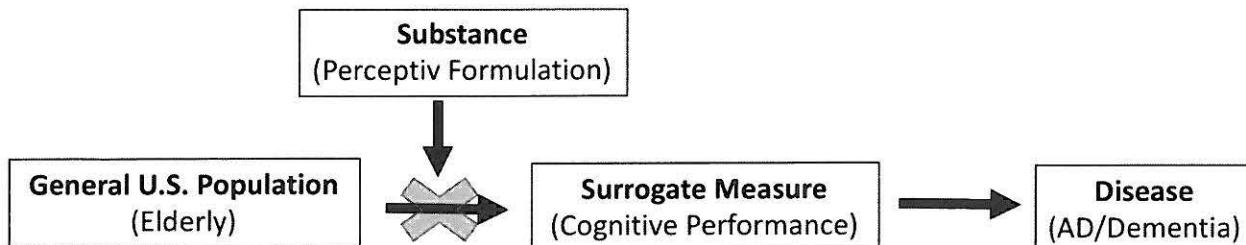


Fig. 4: Diagrammatic representation of the relationship between a surrogate endpoint and the disease: Intake of a dietary substance (the Perceptiv formulation) affects the development of disease (in this case, AD/dementia), and operates, and can therefore be accurately assayed, via an appropriate surrogate endpoint that ultimately affects and clinically defines the development of the disease (in this case, cognitive performance). Modified from Rowlands and Hoadly, 2006.

As will be described in detail in the following section, our adults with no known or suspected cognitive difficulties and individuals with MCI receiving the Perceptiv formulation were followed for periods of 1 year, which was not long enough to make a definitive determination of whether or not there was any alteration in anticipated development of AD; *this would require a longitudinal study spanning a minimum of 5-10 years commencing with individuals ≥50 years of age*. We therefore utilized a surrogate measure as detailed in Rowlands and Hoadly (2006; Fig. 4). In this regard, the *FDA Guidance for Industry: Evidence-Based Review System for the Scientific Evaluation of Health Claims*⁶ specifies:

“Surrogate endpoints are risk biomarkers that have been shown to be valid predictors of disease risk and therefore may be used in place of clinical measurements of the onset of the disease in a clinical trial. Because a number of diseases develop over a long period of time, it may not be possible to carry out the study for a long enough period to see a statistically meaningful difference in the incidence of disease among study subjects in the treatment and control groups.”

Our surrogate measure was improvement of cognitive performance in individuals with no known or suspected cognitive difficulties and improvement or maintenance for individuals diagnosed with MCI, in both cases as an index of risk of developing AD; this is a highly-definitive and appropriate surrogate measure, since, as described above, a critical decline in cognitive performance is the definitive feature that prompts clinical investigation and ultimately defines a diagnosis of MCI and eventual conversion to “probable AD” or “senile dementia of the Alzheimer type,” which may then be corroborated by imaging but can only be confirmed by autopsy (Couto and Millis, 2015). Improvement of cognitive performance clearly reduces the risk of development of diagnosis of AD, since a critical decline in cognitive performance is the defining feature that warrants a provisional diagnosis, which is the only definitive diagnosis pending autopsy. Any individual, not yet diagnosed with “probable Alzheimer’s disease” or “senile dementia of the Alzheimer type,” that has improved in cognitive performance by consuming the Perceptiv formulation would have to undergo a decline in that improvement, followed by a further critical decline to warrant consultation of a physician and potential diagnosis; this increase in the delta of cognitive performance translates into at the very minimum a longer time before such a potential diagnosis. This is further supported by our demonstration that individuals already diagnosed with “probable AD” improved following consumption of the Perceptiv formulation to the extent that their performance qualified as cognitively intact. Accordingly, the measure of cognitive decline is

⁶ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-evidence-based-review-system-scientific-evaluation-health-claims#ftn20>. Last accessed 9/6/2019.

substantially more than a simple surrogate, since a critical decline is the defining feature of a diagnosis of “probable AD” or “senile dementia of the Alzheimer type”.

E. Regulatory Precedent

The FDA has provided precedent for health claims based on consumption of nutritional supplements for (1) Overall neuronal health, (2) Amelioration of cognitive decline and (3) Reduction of risk of dementia in the elderly, all of which are directly relevant to the present petition as follows:

(1) The FDA authorized the health claim that folate (as both folic acid and naturally occurring food folate) reduces the risk of neural tube defects (which are brain and spinal cord birth defects)⁷. This is directly relevant to the present petition since common neural tube defects are derived from lack of sufficient neurons (Mattson and Shea, 2003). Progression of AD is accompanied by loss of neurons (Mattson and Shea, 2003; Rochtus et al., 2015), and long-term daily folic acid supplementation positively impacts cognitive performance and reduces deleterious biomarkers associated with AD (Levine et al., 2015; Kok et al., 2015).

(2) The FDA allowed a qualified health claim for a benefit of phosphatidyl serine for cognitive function with substantially less evidence than is presented herein for Perceptiv⁸.

(3) The FDA also authorized, with caveat, the qualified health claim that phosphatidyl serine can reduce the risk of dementia in the elderly⁷.

III. SUMMARY OF SCIENTIFIC DATA.

We summarize the findings of multiple intervention and observational clinical studies in which participants received the Perceptiv formulation or indistinguishable placebo, including 3 studies with individuals with no known nor suspected cognitive impairment, individuals diagnosed with MCI and individuals diagnosed with AD, published in peer-reviewed scientific journals from 2008 to 2019. Phase I studies were supported by internal funds from the University of Massachusetts Lowell. Phase II studies were supported by a grant to Remington and Shea (the Petitioner) from the national Alzheimer’s Association. Inclusion/exclusion criteria, safety/study-stopping rules, and similar details are included in the publications themselves. No corporate funds were involved in any clinical or preclinical studies, and clinical studies were completed prior to licensing of the Perceptiv formulation.

Types of Studies: We conducted both randomized and observational, prospective cohort studies. In many of our randomized studies, we included observational prospective cohort (referred to as “open-label extensions” in the original publications), in which the cohort originally randomized to placebo was crossed-over to the Perceptiv formulation and were aware of this change (and that they had been receiving the placebo). Simultaneously, the cohort originally randomized to the Perceptiv formulation was maintained on the formulation, but participants were informed that they had received and would continue to receive the formulation. We did not conduct complete “cross-over” of treated and placebo cohorts without breaking code since forced withdrawal of an effective treatment could be considered unethical (e.g., Speigel et al., 2011). While randomized analyses provide the most definitive conclusions, prospective cohort studies

⁷ <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.79>. Last accessed 9/3/2019

⁸<https://www.regulations.gov/document?D=FDA-2002-P-0041-0023>. Last accessed 9/3/2019

are particularly useful for conditions such as cognitive decline, since assigning individuals to a placebo deprives participants of a potentially beneficial intervention for an extended interval, despite the knowledge that early intervention is optimal (Shea and Remington, 2012a,b, 2015; Emery et al., 2001; Soldevila-Domenech et al., 2019). Doing so can be considered unethical (Speigel et al., 2011) by the same line of reasoning as not conducting full cross-over analyses. Accordingly, the usefulness of observational studies for cognitive decline should not be overlooked or minimized. In this regard, we typically observed that, once crossed-over to the Perceptiv formulation, the original placebo cohort demonstrated improvement paralleling that initially displayed by the cohort randomized to the Perceptiv formulation. Furthermore, a cohort of adults with no known or suspected cognitive difficulties demonstrated improvement in an “open-label” prospective observational study that was equivalent to that of a cohort randomized to the formulation (Chan et al., 2010); i.e., despite knowledge of consuming the formulation, this “open-label” did not out-perform a randomized cohort receiving the formulation under blind conditions.

Analyses of Data: While we relied on *t* tests and ANOVA in laboratory studies presented herein, in our clinical studies, we also calculated Cohen’s Clinical Effect Size at each time interval [according to the formula: [(mean at treatment time) – (mean at baseline)] / standard deviation of baseline of entire participant pool, with values >0.2 considered clinically-significant] and/or [(mean of treated cohort) – (mean or placebo cohort) / standard deviation of baseline of entire participant pool] (Chan et al., 2010; Remington et al., 2015a,b). Clinical Effect Size quantifies the size of the difference between two experimental groups and/or between a group’s performance after versus before an intervention and may therefore be a more precise method to determine the clinical importance of any difference (Coe, 2002). A major reason behind this is that a statistically significant result ($p<0.05$) derived from a *t* test or ANOVA is derived from both the magnitude of a change and the size of the population in an inseparable manner. Significance can be derived from a large change with a small-modest sample population, but also from a small change with a large sample population; in the latter case, the change, although significant, can very well have no clinical impact on participants (Coe, 2002). Clinical Effect Sizes are useful for comparison among studies and for evaluating whether or not interventions are clinically meaningful, especially in neuropsychology studies (Fritz et al., 2012; Morris and Fritz, 2013; Cummings et al., 2017; Bezeau and Graves, 2001; Peters, 2013; Schafer and Schwarz, 2019). Importantly, while a statistically significant finding that does not have clinical significance may have little to no benefit, it has also been pointed out that a clinically significant result that does not also display statistical significance may be derived from chance alone (El-Masri, 2016). Accordingly, we consider statistical and Clinical Effect analyses both of importance in evaluation of interventions to maintain or improve cognitive performance and to reduce the risk of cognitive decline. We further relied on paired *t* tests for our studies. This was derived from the likelihood that each individual’s baseline cognitive performance and respective change in performance over time varied considerably; we therefore considered that comparison of cohort performance following randomization versus respective baseline (via paired *t* tests) was more informative than comparison of collective performance via unpaired *t* tests. However, to facilitate review and evaluation, we also calculated and include unpaired *t* tests herein.

A. Clinical Trials with Individuals with no Known or Suspected Cognitive Difficulties Intervention Studies

•**Study 1:** Chan et al. (2010) conducted a 3-month, multi-site, randomized, placebo-controlled trial with 138 individuals aged 18-86 years of age that had no known or suspected cognitive difficulties. Participants were randomized to the Perceptiv formulation or indistinguishable placebo and completed the California Verbal Learning Test and the Trail-Making Test (parts A and B). While we considered this initially as a Phase I study in our IRB application, the degree of efficacy of the Perceptiv formulation with multiple tests and the number of participants, along with

the prior publication of two Phase I trials (Chan et al., 2008a; Remington et al., 2009), give Chan et al. (2010) the caliber of a Phase II trial.

The California Verbal Learning Test ("CVLT"; version II) consisted of repeating a list of 10 words immediately after hearing them, 5min after hearing them and repeating a different word list during this interval as a distractor ("Short-term" recall) and an additional 20min after hearing them with a non-verbal task conducted during this interval as a further distractor. These data were reported in separate graphs in Fig. 1 of Chan et al. (2010). Herein, we present these data on a single graph which contains a time-line orientation of performance on Immediate, Short-term and Long-term recall (Fig. 5), which may facilitate comparison of cohort performance with regard to time. Cohorts randomized to the Perceptiv formulation and placebo performed identically in immediate recall, demonstrating that both cohorts heard the word list clearly and had an identical ability to recall words in the list immediately. By contrast, the cohort randomized to the placebo declined in Short-term recall versus the cohort randomized to the Perceptiv formulation ($p=0.03$, Student's t test); the cohort randomized to the placebo declined by 66% (which also differed statistically from their own immediate recall; $p= 0.01$), while the cohort randomized to the Perceptiv formulation declined by only 14% (which did not differ statistically from their own immediate recall; $p=0.32$, Student's t test). Performance of both groups declined in Long-term recall; the cohort randomized to the Perceptiv formulation performed slightly but not significantly better than the cohort randomized to the placebo. Accordingly, Chan et al. (2010) concluded that consumption of the Perceptiv formulation for 3 months improved short-term recall.

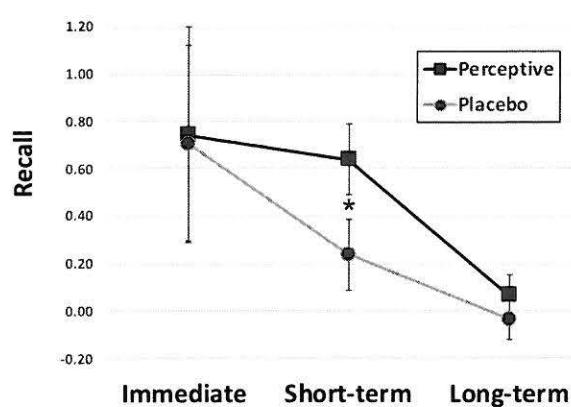


Fig. 5: Performance on the CVLT of individuals 18-86 years of age randomized to the Perceptiv formulation or indistinguishable placebo for 3 months. Data are re-graphed from Chan et al. (2010) to generate a timeline of Immediate, Short-term and Long-term responses; the distances between these points along the x axis are arbitrary and are intended to indicate simply the order of events rather than the actual time between events. "Immediate" presents the mean (\pm standard error of the mean) result of participants repeating a list of 10 words immediately after hearing them; this portion of the test is carried out 4 times according to standard instructions provided for the CVLT; the average of 4 times is graphed herein to place it on the same scale as the next two measures. "Short-term" presents the result of participants repeating the same list after a 5min interval during which participants were asked to repeat a different list as a distractor. "Long-term" presents the result of participants repeating the same list after an additional 20min interval during which they carried out a nonverbal task as a distractor (in our studies, the Trail-making test was used as the long-term distractor). Participant scores are adjusted according to the CVLT manual for age and education. The asterisk indicates a significant improvement in recall for participants receiving the Perceptiv formulation versus those receiving placebo ($p = 0.031$; Student's t test).

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Participants also completed the Trail-making test (parts A and B). As described in Chan et al. (2010), participants are asked to connect a series of numbers (1-25) in increasing order (Trails A). This was followed by Trails B, in which participants were asked to connect a series of alternating numbers and letters in order (1, A, 2, B, 3, C, 4, D, etc.). Participants completed a short sample of Trails A and B prior to each respective test to confirm comprehension of instructions prior to commencing; this sample was not timed or factored into participant performance. The length of time for completion of each test was recorded. Performance on both portions of this test is subject to a variety of impairments including difficulties in neuromuscular

coordination, vision and following simple instructions. However, Trails B also incorporates executive function due to the need to alternate between numbers and letters. In efforts to isolate the impact of consumption of the Perceptiv formulation on executive function, each participant's score on Trails A is subtracted from that on Trails B at each interval to yield a "B-A" score; this is a standard approach for this test instrument. Since performance on both components of the Trail-making test is also subject to age and education of the participant, individual scores were normalized according to age and education level of the participant according to the normative scores presented in Tombaugh (2004) prior to subtracting respective scores on Trails A from Trails B. The change between baseline and 3 months in Trails B-A was determined for each participant by subtracting baseline values from their respective 3-month values.

During normalization of scores, we also compared the performance of age ranges specified in Tombaugh (2004). As described in Chan et al. (2010), we noted that participants aged 30-49, 50-64 and 64-73 displayed identical improvement following 3 months of consumption of the Perceptiv formulation (see Fig. 5 in Chan et al., 2010). However, the average score for participants ≥ 74 years of age was significantly worse. Closer examination revealed that a small number of individuals >74 years of age presented substantially declined in performance on the Trail-making test (by 42-146 seconds) at 3 months versus their respective baseline performance. Notably, this level of decline was not typical of the total cohort of 27 participants ≥ 74 years of age. Moreover, this decline was specifically in part B of the test. A similar extent of decline was not observed in part A (in which participants connect circles containing only numbers) and therefore is based on simple memory and vision, hand-eye coordination and neuromuscular control. These findings indicate a specific decline in executive cognitive performance rather than age-related decline in simple memory and/or physical/motor function. As described in Chan et al., this left us with concerns regarding potential deficiencies in basal nutrient levels and/or supplement adsorption by the elderly. For simplicity and to expedite dissemination of our findings, we elected to make no overall conclusion regarding participants >74 years of age at that time. The findings presented in Chan et al. (2010; summarized in Table 3) were therefore confined to the 93 participants ≤ 74 years of age.

Table 3: Chan et al. (2010) participant demographics and performance on the Trail-Making Test

		Treated	Placebo	<i>p</i>
	n	51	42	
Gender	% Female	41%	45%	
Age	Range	18-74	18-74	
	Mean \pm SD	57.9 \pm 15.1	61.6 \pm 13.6	
Education	% \geq College	43	31	
Trails B-A				
Baseline	Mean \pm SD	2.6 \pm 28.8	4.7 \pm 20.7	0.7
3 months	Mean \pm SD	-7.4 \pm 14.4	3.1 \pm 29.5	0.04

Values present participant demographics and performance of participants ≤ 74 years of age on the Trail-Making Test (scores for Trails B-Trails A) at baseline and following 3 months of receiving the Perceptiv formulation (Treated) or Placebo for total participants and those participants stratified according to age (both age range as well as mean age \pm standard deviation are presented). "SD" indicates standard deviation. "% \geq College" indicates the percentage of the cohort that had completed 4 years of college or more. Performance values are mean \pm standard deviation (SD). "P" indicates *p* values comparing Treated vs Placebo cohorts via 1-tailed Student's *t* test. Table modified from Shea and Remington, 2019.

Chan et al. (2010) demonstrated that participants ≤ 74 years of age receiving the Perceptiv formulation (referred to as “Treated” for simplicity of writing) improved statistically ($p < 0.03$) and clinically (Effect Size = 0.84) in the Trail-making test within 3 months versus their baseline performance, while those receiving an indistinguishable placebo did not improve. We did not include statistical comparison of performance of Treated vs. Placebo cohorts in Chan et al. (2010) but did so in our more recent publication (Shea and Remington, 2019); comparison of Treated and Placebo cohorts via *t* test indicated that they were statistically-identical at baseline but that the Treated cohort demonstrated statistically-significant improvement versus the Placebo cohort after 3 months of randomized treatment (Table 3; modified from Shea and Remington, 2019).

Since it was noted that those individuals > 74 years of age that *did* improve displayed the same extent of improvement as younger individuals (Chan et al., 2010; see also Shea and Remington, 2018b), we examined more closely the differences in performance of the “elderly” (> 74 years of age; also referred to as “aged”) versus “younger” (≤ 74 years of age) cohorts (Shea and Remington, 2018a, b). The change in performance for individuals ≤ 74 years of age after 3 months of treatment ranged from -38 to +38 seconds, while for those > 74 years of age ranged from -38 to +146 seconds (a 2.4x greater range compared to that of individuals ≤ 74 years of age). Following randomization, the treatment and placebo cohorts ≤ 74 years of age were statistically identical in terms of gender, education level and baseline scores on the Trail-making test. By contrast, the treatment and placebo > 74 years of age were identical in terms of gender and education level, but baseline scores on the Trail-making test were statistically lower in the cohort randomized to the Perceptiv formulation versus placebo. As described in Chan et al., those individuals > 74 years of age that were classified as “responders” (i.e., who improved in the Trails test) improved to the same extent as participants ≤ 74 years of age, indicating that neither the significant decline of the participants listed above nor the wide range in baseline scores were characteristic of the entire cohort of aged participants. Shea and Remington (2019) furthermore combined the performance of previously omitted individuals > 74 years of age with those of the previously published individuals ≤ 74 years of age (excluding those previously considered as potential outliers) into single Treated and Placebo cohorts (18-85 and 18-86 years of age, respectively). When this was carried out, the now-expanded Treated and Placebo cohorts demonstrated statistically identical performance on the Trail-Making Test at baseline, but the Treated cohort demonstrated statistically significant improvement versus that of the placebo cohort after 3 months (Table 4).

Table 4: Total participant demographics and performance of participants from Chan et al. (2010) on the Trail-Making test

Total Participants			
	Treated	Placebo	
n	72	64	
Gender	% Female	32%	31%
Age	Range	18-85	18-86
	Mean \pm SD	67 \pm 12.9	68.4 \pm 12.0
Education	% \geq College	38	48
Trails B-A			p
Baseline	Mean \pm SD	-0.4 \pm 27.1	3.1 \pm 24.0
3 months	Mean \pm SD	-7.8 \pm 15.4	1.5 \pm 27.3
			0.02

Values present demographics and performance of participants from Chan et al. (2010) on the Trail-Making Test (scores for part B - part A) at baseline and following 3 months of receiving the Perceptiv formulation (Treated) or Placebo for total participants (both age range as well as mean age \pm standard deviation are presented). Data are from Chan et al., 2010 and Shea and Remington, 2019. "SD" indicates standard deviation. " \geq College" indicates completion of 4 years of college or more. Performance values are mean \pm SD. "P" indicates p values derived by comparison of deltas of Treated vs Placebo cohorts via 1-tailed Student's t test. Table modified from Shea and Remington, 2019.

It has been argued, with merit, that removal of outliers can increase the likelihood of Type 1 errors and/or compromise the null hypothesis (e.g., Bakker and Wicherts, 2014). We note, however, that based on the wide range of scores of >74 aged cohort in Chan et al. (2010), this cohort likely consists of a diverse group of individuals with respect to cognitive performance: some with cognitive performance considered normal for their respective age, some with impending dementia, and, as we have shown by consideration of outliers, some who underwent demonstrable decline in performance during our analyses. Since these participants self-reported no known nor suspected cognitive decline, their respective cognitive difficulties likely did not pervade day-to-day performance and were revealed only by part B of the Trail-making test, which quantifies executive function. In support of this possibility, the individuals displaying extreme decline in part B of the Trail-making test did *not* display a similar decline in part A (which quantifies simple memory) or on the CVLT (which assays simple word recall; Chan et al., 2010). This possibility is further supported by the lack of a physician's diagnosis confirming normal cognitive performance for their respective age; we did not require a physician's diagnosis as an inclusion criterion but rather relied on self-reporting.

This differential performance between younger and older cohorts in Chan et al. is consistent with the frequency of AD; clinical cognitive decline is rare among individuals <65 years of age, is present in 3% of individuals 65-74 years of age, and present in 17% of individuals 75-84 years of age ([alz.org/facts-and-figures.pdf](https://www.alz.org/facts-and-figures.pdf)⁹). In this regard, 17% of the 27 participants >74 years of age correlates with the 5 participants who underwent marked decline in executive function (Shea and Remington, 2018a, 2019).

This above 3-month randomized study was followed by a 9-month open-label extension with the same participants in which the former placebo cohort was crossed over to the Perceptiv formulation. The randomized and open-label extension are presented in a single graph within Chan et al. (2010); however, for this petition, we will describe the open-label extension below under Observational Studies.

•Study 2: In efforts to determine whether or not consumption of the Perceptiv formulation for a full 3 months was required for individuals with no known or suspected cognitive difficulties to improve in cognitive performance, Chan et al. (2010) also conducted a shorter randomized, placebo-controlled trial during which an additional 43 participants received the Perceptiv formulation or placebo under double-blind conditions for 2 weeks. Cohorts randomized to the Perceptiv formulation or placebo were statistically identical in gender, age and baseline performance on the Trail-Making Test and the Digit-Recall test (Table 5).

⁹ <https://www.alz.org/media/homeoffice/facts%20and%20figures/facts-and-figures.pdf>. Last accessed 8/28/2019

Table 5: Participant demographics for the 2-week randomized Trail-making test

	Treated	Placebo	p value
Number of Participants	24	17	
Gender (% F)	64	82	0.24
Age Range	22-79	21-80	
Mean Age (\pmSD)	54 (16)	49 (19)	0.33
Baseline Trails (Mean \pmSD)	-7.4 (18.0)	-8.5 (17.0)	0.85
Baseline Digit-Recall	55.9 (29.3)	49.5 (23.9)	0.26

As shown within Chan et al. (2010), participants receiving the Perceptiv formulation statistically improved statistically ($p<0.05$) versus their respective baseline, while those receiving the placebo did not improve. We did not include statistical comparison of the cohort receiving the Perceptiv formulation versus the cohort receiving placebo in Chan et al., (2010); however, we include that comparison herein. Appendix C contains participant performance to facilitate review and evaluation, and demonstrates that the cohort receiving the Perceptiv formulation improved statistically versus the cohort receiving placebo within 2 weeks ($p<0.03$; Appendix C) within 2 weeks.

The same participants completed the Digit-Recall Test (also known as the Digit-Memory test), in which they were asked to repeat a series of numbers, beginning with 2 numbers and increasing up to 8 numbers (with different numbers in each series); this was continued until they made an error or a series of 8 numbers was correctly repeated. They were then asked to repeat new series of numbers, again starting with a series of 2 and increasing up to a series of 8 (distinct from each other and from the first set), but were asked to repeat them in reverse order. The total number of correct responses was normalized according to age and percentiles (available at <http://www.dyslexiaaction.org.uk/Administration/uploads/Digit.pdf>). As shown within Chan et al. (2010), participants receiving the Perceptiv formulation improved statistically ($p=0.05$) versus their respective baseline, while those receiving placebo did not improve. We did not include statistical comparison of the cohort receiving the Perceptiv formulation versus the cohort receiving placebo in Chan et al., (2010); however, we include that comparison herein. Appendix C contains participant performance to facilitate review and evaluation, and demonstrates that the cohort receiving the Perceptiv formulation improved statistically versus the cohort receiving placebo within 2 weeks ($p= 0.05$; Appendix C).

This 2-week randomized study was followed by a 2-week open-label extension in which the former placebo cohort was crossed over to the Perceptiv formulation. The randomized portion and open-label extensions for these tests are presented in single graphs within Chan et al. (2010); however, for this petition, we will describe the open-label extension below under Observational Studies.

Observational (Prospective Cohort) Studies

•**Study 1:** Chan et al. (2010) continued with participants in the above randomized 3-month study in a 3-month open-label extension, in which the Perceptiv formulation was provided to all participants (including the former placebo cohort) and all participants were aware of what they received. All participants improved over this 3-month extension; the cohort initially receiving Perceptiv demonstrated further statistical ($p<0.01$) and clinical (Effect Size = 1.1) improvement, while the former placebo cohort improved to a level statistically identical to that of the cohort originally receiving Perceptiv (Chan et al., 2010); this was to be expected since the delayed-start group has now received the formulation for 3 months.

Chan et al. then conducted a second phase of this observation study, in which they withdrew the formulation from all participants for 3 months; this phase could technically be presented herein as a separate study, but instead is presented as a continuum with the first portion of observational study as in the original publication. After 3 months in the absence of the Perceptiv formulation, cognitive performance of all participants declined to their original baseline (which also confirmed that prior cognitive improvement was not due familiarity with test instruments). After this “wash out” period, Chan et al. once again provided the Perceptiv formulation for 3 months to all participants under open-label conditions, after which participants once again demonstrated statistical improvement versus baseline performance and post “wash out” performance. Throughout these observational analyses, participants were maintained in the original treated and placebo cohorts and presented as such to facilitate comparison of performance.

Notably, performance of both the original Treated and Placebo cohorts increased following consumption of the Perceptiv formulation for 3 months both times under open-label conditions to the same extent as that of the original Treated cohort in the initial randomized portion of this year-long study (Chan et al., 2010).

•**Study 2:** Chan et al. (2010) continued with participants in the above randomized 2-week study in a 2-week open-label extension, in which the Perceptiv formulation was provided to all participants (including the former placebo cohort) and all participants were aware of what they received. Participants initially receiving Perceptiv continued to improve during the 2-week open-label extension in both the Trail-Making and Digit-Recall Tests. Once crossed over to Perceptiv, those initially receiving placebo improved statistically and clinically versus their respective performance while receiving placebo. While the cohort receiving the Perceptiv formulation had improved statistically versus the cohort receiving the placebo by the end of the initial randomized 2-week portion of in both Tests, at the end of the 2-week open label extension, the initial placebo cohort, now also receiving the Perceptiv formulation, improved to the extent that their performance on both Tests was now identical to that of the cohort originally receiving the Perceptiv formulation (Appendix C).

•**Study 3:** Chan et al. (2010) conducted a 6 month study with a separate cohort of 38 individuals with no known or suspected cognitive difficulties who received the Perceptiv formulation under open-label conditions (i.e., were assigned to the formulation and were aware of it); notably, this cohort demonstrated statistical ($p<0.04$) and clinical (Effect Size = 0.84) improvement in cognitive performance within 3 months. *Of interest, the extent of improvement of this open-label cohort was identical to, rather than greater than, that displayed within the first 3 months by the above group that had been randomized to the Perceptiv formulation; this dispels potential concerns that the knowledge that an individual is consuming the Perceptiv formulation would result in cognitive improvement solely due to a “placebo effect.”*

B. Clinical Trial with Individuals Diagnosed with MCI Intervention Study

•**Study 1:** Remington et al. (2015a) conducted a 6-month, randomized, placebo-controlled trial with 34 individuals recruited from two sites, who were 65.9 ± 11.3 years of age with 14.5 ± 2.4 years of education, and had been diagnosed with MCI. Participants completed the Clock-Drawing test, which assesses executive function (Royall et al., 1998); participants are asked to draw the face of an analog clock, place the numbers within the face, draw a large and small hand (to indicate minutes and hours) with arrows on the hands, and to place the hands to indicate a specified time (in this case, “12:45”). Participants also completed the Dementia Rating Scale

(DRS), which assesses a variety of cognitive performance across a number of domains from simple to complex such as memory, attention and conceptualization (Jurica et al., 2001).

Following randomization, the Treated and Placebo cohorts were statistically identical in age, education and baseline performance on the Clock-drawing test and both raw and age- and education-adjusted baseline scores on the DRS (Table 6; see also Remington et al., 2015a).

Table 6: Demographics of Participants Diagnosed with MCI Following Randomization

	Treated	Placebo
Participant number	22	12
Age (years)	63 ± 12	61 ± 11
Education (years)	15 ± 1.4	14 ± 3
Baseline Clock-Drawing test	13.9 ± 1.5	13.2 ± 2.3
Baseline DRS (raw scores)	138.2 ± 7.4	133.4 ± 12.5
Baseline DRS (AEMSS adjusted) *	11 ± 4	10 ± 4.2

*refers to the “Age and Education Adjusted Moans Scale Score” (AEMSS; a standard approach defined in the DRS manual that normalizes performance among individuals based on relative age and relative extent of education (Jurica et al., 2001).

As shown in Remington et al. (2015a), participants randomized to the Perceptiv formulation displayed a significant increase in performance on the DRS within 3 months (Effect Size = 0.76), continued to improve by 6 months (Effect Size = 0.79) and maintained this level of improvement for the duration of the study. Participants receiving the placebo did not improve on the DRS. Participants randomized to the Perceptiv formulation maintained their baseline performance on the Clock-Drawing test, while those randomized to placebo declined, yielding a difference between these cohorts such that the Treated cohort performed clinically better than the Placebo cohort (Effect Size = 0.34)

We did not include statistical comparison via Student's *t* test between Treated and Placebo cohorts in our original presentation (Remington et al., 2015a) but include these comparisons herein. Due to the large variance in performance, we did not observe a significant difference in the DRS in either paired or unpaired *t* tests. After 6 months (the length of the randomization period), we observed a significant difference ($p = 0.045$) in the Clock-Drawing test between the Treated and Placebo cohorts. Notably, as with Effect size (above), this difference was derived from *maintenance* of baseline performance of the Treated cohort ($p = 0.351$; Treated cohort after 6 months versus baseline), compared to a statistical *decline* in performance of the Placebo cohort ($p = 0.038$, Placebo cohort after 6 months versus baseline). Since these values were not previously published, we include them in Appendix D to facilitate review and evaluation.

We recently compared the performance of Treated versus Placebo cohorts during the course of treatment more closely by segregating participants into previously established categories according to their performance on the DRS (Shea and Remington, 2019). The DRS manual provides classification of participant performance as intact (scoring ≥ 9), mildly impaired (6-8), moderately impaired (4,5), and severely impaired (≤ 3 ; Jurica et al., 2001). Participants were separated into overall cohorts demonstrating “intact” (≥ 9) or “impaired” (≤ 8) performance on the DRS after 3 months. This parallels the distinction of Early MCI versus Late MCI based on subtle differences in performance on standard cognitive tests (Jessen et al., 2014). This classification highlighted that the above statistically-significant decline in performance for the Placebo cohort on the Clock-Drawing test was confined to those classified as impaired on the DRS; individuals

classified as intact on the DRS displayed performance on the Clock-Drawing test that was statistically identical to baseline values (Fig. 6; Shea and Remington, 2019).

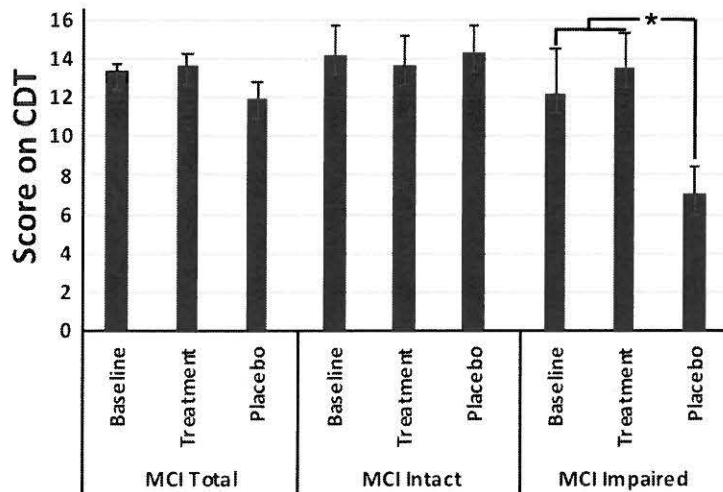


FIG. 6: Comparison of Total Participants Diagnosed with MCI, Those Classified as Intact and Those Classified as Impaired Values represent the mean \pm standard error of performance on the Clock-Drawing Test (CDT) at baseline and after receiving the Perceptiv formulation (Treatment) or placebo for 3 months for all participants (total) and those participants stratified as “Intact” or “Impaired” according to their respective performance on the DRS. Asterisk indicates $p<0.05$; Student’s t test. From Shea and Remington, 2019.

It should be noted that participants were close to the maximum possible score on the Clock-Drawing Test (15 maximal points), leaving little room for improvement from baseline, but highlighting the significant decline for those classified as impaired according to the DRS.

These findings underscore that nutraceutical intervention may not impart apparent improvement for individuals with initial high-level functioning but may nevertheless be involved in maintenance of existing levels of cognitive performance (Shea and Remington, 2019).

This intervention study was followed by a followed by a 6-month observational “open-label extension” in which the former placebo cohort was crossed over to the Perceptiv formulation as described in the following section.

Observational (Prospective Cohort) Study

•**Study 1:** As described above, Remington et al. (2015a) continued with participants diagnosed with MCI from one of the sites in the above randomized 6-month study by conducting a 6-month open-label extension, in which the Perceptiv formulation was provided to all participants (including the former placebo cohort) and all participants were aware of what they received. During this observational extension, the Treated cohort maintained the clinical improvement it had attained during the randomized period. The original placebo cohort improved during this observational extension in the DRS such that they displayed an Effect size of 0.35 and 0.22 at 3 and 6 months, respectively, of this extension (corresponding to time points 9 and 12 months for the entire study) versus their own baseline (time 0; Remington et al., 2015a). Both the Treated and original Placebo cohorts maintained their baseline performance in the Clock-drawing Test during this observational extension. *These findings indicate long-term efficacy of the Perceptiv formulation.*

C. Clinical Trials with Individuals Diagnosed with Alzheimer's Disease

Inclusion of the following clinical studies for individuals diagnosed with AD are relevant in accord with FDA guidelines since the Perceptiv Formulation improves or maintains cognitive performance by the same mechanism for individuals diagnosed with AD as for individuals with MCI or no known or suspected cognitive difficulties. We elaborate on this point in section E, following presentation of our findings for individuals diagnosed with AD.

Intervention Studies

•**Study 1:** Remington et al. (2015b) conducted a Phase II, 3 month, multi-site, randomized, placebo-controlled trial with 106 individuals diagnosed with “probable Alzheimer's disease” or “senile dementia of the Alzheimer type”, followed by a 9-month open-label extension in which the former placebo cohort was crossed over to the Perceptiv formulation; the open-label extension will be discussed below under “Observational Studies”.

The formulation was highly effective. Participants randomized to the Perceptiv formulation improved statistically versus those randomized to the placebo within 3 months in the Clock-Drawing test ($p = 0.0083$) and the AEMSS-adjusted DRS ($p = 0.0266$; Remington et al, 2015b). Participants randomized to the Perceptiv formulation displayed statistical ($p < 0.01$) and clinical (Effect size = 0.24) improvement in cognitive performance on both the Clock-Drawing test and the AEMSS-adjusted DRS within 3 months versus their own baseline performance. The placebo cohort did not improve in cognitive performance on either test.

Dedicated caregivers reported on Behavioral and Psychological Symptoms of Dementia (BPSD) of their respective participants by completion of the standard NeuroPsychiatric Inventory (NPI). Neither cohort displayed statistical or clinical change from baseline in total NPI, nor did they differ statistically from each other. Participants receiving the Perceptiv formulation displayed a 1.86-fold improvement in total NPI versus those receiving placebo at 3 months; however, caregiver reports for both cohorts displayed considerable variance as reflected by large standard errors (-1.5 ± 8 versus -0.8 ± 1 , Treated and Placebo cohorts, respectively), which may have precluded significance. Notably, caregiver evaluation, though quantified, remains subjective.

Caregivers reported a trend towards statistical improvement ($p < 0.06$) versus the individual's own baseline in depression (one domain assayed by the NPI) for the Treated cohort and a trend ($p = 0.06$) toward statistical improvement in the motor domain for the placebo cohort. Fewer appetite abnormalities were reported for the Treated versus the Placebo cohort (-0.02 ± 1.1 vs. -0.4 ± 1.5 , respectively). Caregivers did not report statistical or clinical change in other domains of the NPI. Caregivers reported no change in Activities of Daily Living (a test instrument which assays common daily functions such as dressing, using appliances, etc.) for either cohort.

This 3-month randomized study was followed by a 9-month open-label extension with the same participants in which the former placebo cohort was crossed over to the Perceptiv formulation (Remington et al., 2015b); for this petition, we will describe the open-label extension below under Observational Studies.

•**Study 2:** Remington et al. (2009) conducted a Phase I 3-month randomized, placebo-controlled trial with 12 residents of nursing homes diagnosed with moderate-severe Alzheimer's disease. While all participants declined in cognitive performance over the 9-month trial, the cohort receiving Perceptiv demonstrated a clinically significant (Effect size = 0.38) attenuation in the rate of decline versus that of the cohort receiving placebo. The cohort receiving the Perceptiv formulation displayed a 30% improvement in BPSD over the 9-month treatment period, while the

cohort receiving placebo declined; this difference was not statistically or clinically significant.

Observational (Prospective Cohort) Studies

•**Study 1:** During the 9-month open-label extension of randomized study 1 (above), the original Perceptiv cohort continued to improve in cognitive performance and maintained their baseline performance in BPSD. Following crossover to the Perceptiv formulation, the original placebo cohort improved in cognitive performance to the same extent as the original treatment cohort and returned to their baseline performance in BPSD (Remington et al., 2015b). No serious adverse events were reported.

•**Study 2:** Remington et al. (2016) conducted a 12-month open-label trial with 24 community-dwelling residents diagnosed with probable Alzheimer's disease or senile dementia of the Alzheimer type. This cohort was comprised of individuals who initiated consumption of this formulation on their own or via caregiver/physician advice after publication of phase I studies (Chan et al., 2008a; Remington et al., 2009) and who requested participation in Study 1 (above). Since randomization of these individuals could result in temporary forced withdrawal of any benefit of the Perceptiv formulation, they were provided the Perceptiv formulation with IRB approval under open-label conditions for 12 months. These participants maintained their baseline cognitive performance and BPSD during these 12 months. Remington et al. also demonstrated that these findings contrasted directly with the routine decline in cognitive performance and BPSP over 6-12 months observed for multiple historical placebo cohorts from multiple prior published studies.

•**Study 3:** Chan et al. (2008a) conducted a Phase I, 12-month, open-label trial with 14 community-dwelling individuals diagnosed with mild-moderate Alzheimer's disease, followed by a 16-month extension. Notably, since this was the first clinical study with the Perceptiv formulation, Chan et al. elected an open-label design both for safety concerns and to foster regarding compliance over the proposed 12-month trial. Participants demonstrated clinical (Effect size = 0.35) and statistical ($p<0.02$) improvement in cognitive performance within 6 months and maintained this improvement until 12 months (the length of the original study period). Participants also demonstrated clinical (Effect size = 0.36) and statistical ($p<0.02$) improvement in BPSD within 6 months and maintained that improvement until 12 months. After these 12 months, a subset of caregivers requested continuation of treatment. With IRB approval, Chan et al. therefore conducted a 16-month, non-invasive extension, during which caregivers continued to report on BPSD, but participants were not asked to continue cognitive tests. Participants maintained the above clinical improvement in BPSD for the duration of this 16-month extension, which represents a total treatment time of 28 months.

D. Summary of and Rating of Clinical Studies with the Perceptiv formulation

The above clinical studies are included in Appendix E, as well as in the list of total citations for this document. These studies are summarized in Table 7.

Table 7: Summary of Clinical Studies with the Perceptiv formulation

Participant Diagnosis	Randomized, placebo controlled	Length of study (months)	Statistical/Clinical Improvement		Placebo Cross-over	Placebo Statistical/Clinical Improvement after crossover
			Cognitive Performance	BPSD*		
Normal Cognition ¹⁻⁴	Yes	12	Yes	n/a	3 months	Yes
Normal Cognition ¹	Yes	1	Yes	n/a	2 weeks	Yes
Normal Cognition ¹	No open label	6	Yes	n/a	n/a	n/a
MCI ^{4,5**}	Yes	12	Yes	n/a	6 months	Yes
	Yes	12	Yes	n/a	n/a	n/a
AD all stages ⁶	Yes	12	Yes	Yes	3 months	Yes
AD all stages ⁷	No open label	12	Maintained	Maintained	n/a	n/a
AD mild-moderate ⁸	No open label	12	Yes	Yes	n/a	n/a
AD moderate-severe ⁹	Yes	9	Yes	No	No	n/a

*Behavioral and Psychotic Symptoms of Dementia

¹Chan et al. (2010) Efficacy of a vitamin/nutraceutical formulation on cognitive speed and recall in adults with no known or suspected dementia. J Nutri Health Aging 14: 224-230.

²Shea TB, Remington R. (2018a) Apparent cognitive decline as revealed by an executive function test within a cohort of elderly individuals self-reporting normal cognitive performance. J Alz dis: 61: 913-915

³Shea TB, Remington R. (2018b) Cognitive improvement in healthy older adults can parallel that of younger adults following lifestyle modification: support for cognitive reserve during aging. J Alz dis Reports 2: 201-205

⁴Shea TB and Remington R. (2019) Relative efficacy of a nutraceutical formulation on cognitive performance across the adult lifespan and during early cognitive decline. J. Alz dis Reports 3: 251-255. DOI: 10.3233/ADR-190124

⁵Remington et al. (2015a) A nutritional formulation for cognitive performance in Mild Cognitive Impairment: a placebo-controlled trial with an open-label extension. J Alz dis: 48:591-595.

^{**}Included a cohort with crossover at 6 months and, as described herein, a cohort for which randomization was maintained for 12 months.

⁶Remington et al. (2015b) A nutritional formulation for cognitive performance and mood in Alzheimer's disease: a phase II multi-site randomized trial with an open-label extension J Alzheimer's dis 45:395-405.

⁷Remington et al. (2016) Maintenance of cognitive performance and mood for individuals with Alzheimer's disease following consumption of a nutraceutical formulation: a one-year, open-label study. J Alz dis: 51: 991-995

⁸Chan et al. (2008a) Efficacy of a vitamin/nutraceutical formulation for early-stage Alzheimer's disease: A one-year open-label pilot study with a 16-month extension. Am J Alz Dis Other Dementias 23: 571-585.

⁹Remington et al. (2009) Efficacy of a vitamin/nutraceutical formulation for moderate to late-stage Alzheimer's disease: A placebo-controlled pilot study. Am J Alz Dis Other Dementias 24: 27-33.

While Perceptiv is not a drug and we do not seek drug status, we note that our clinical studies for individuals with AD and MCI are in compliance with the directive to include outcome measurements on "...both a cognitive and a functional or global assessment scale..." as described in the FDA's "Guidance for Industry. Alzheimer's disease: Developing Drugs for the Treatment of Early Stage Disease."¹⁰

¹⁰<https://www.federalregister.gov/documents/2018/02/16/2018-03226/early-alzheimers-disease-developing-drugs-for-treatment-draft-guidance-for-industry-availability>. Last accessed 9/8/2019.

Moreover, all of the above studies encompassed the surrogate measure of cognitive decline; the Perceptiv formulation improved cognitive performance for individuals with no known cognitive difficulties and for individuals diagnosed with MCI, and furthermore improved cognitive performance for individuals diagnosed with AD.

Notably, effect sizes observed for the Perceptiv formulation in randomized studies for individuals diagnosed with AD (0.24 for executive function; Remington et al., 2015b) matched those reported for and approved for clinical use for the high dose of cholinesterase inhibitors (Rockwood, 2004; Raina et al., 2008). This effect size also matched the effect sizes reported for the medical food Souvenaid (0.21 and 0.20) for individuals diagnosed with mild AD (Cummings et al., 2019a). Effect size on executive function for the Perceptiv formulation for individuals diagnosed with MCI (0.34; Remington et al., 2015a) exceeded the above agents and the mean effect sizes of 0.27 of 0.24 on executive function following meta-analysis of 17 studies with cognitive intervention for individuals diagnosed with MCI (Li et al., 2011). While we find no direct comparison of effect sizes for supplementation for adults with no known or suspected cognitive difficulties in literature searches, it is notable that effect sizes in our randomized studies for executive function for adults with no known or suspected cognitive difficulties were substantially larger than for those diagnosed with MCI or AD (0.84; Chan et al., 2010).

E. The Perceptiv Formulation Improves or Maintains Cognitive Performance by the Same Mechanism for Individuals Diagnosed with AD as for Individuals with MCI or no Known or Suspected Cognitive Difficulties

It is imperative to note that a “diagnosis of AD” or “conversion to AD” as stated in many publications, and reiterated above, is actually shorthand for the true diagnosis of “probable AD” or “senile dementia of the Alzheimer type”. As described above, cognitive performance remains the sole index leading to a diagnosis of “probable AD” or “senile dementia of the Alzheimer type” which can only be confirmed or denied as actual AD by autopsy. Moreover, an individual’s cognitive performance is assessed by standard, vetted cognitive tests, including those used in our studies. Notably, a variance of a single point, resulting from a single additional incorrect answer, can define the difference between normal and impaired cognitive performance. As described above, impairment in performance on standard tests without compromise in normal daily functioning can warrant a diagnosis of MCI. Only minor differences in performance on standard cognitive tests (≤ 1 standard deviation below normative scores) dictates whether one is characterized as having Early versus Late MCI (Jessen et al., 2014). Further impairment such that normal daily activities are compromised can warrant diagnosis of AD. Notably, cognitive tests demonstrate that some aspects of cognitive decline, including memory, reasoning, spatial visualization, processing speed and executive function, begin when individuals are 20-30 years of age and continue throughout life (Salthouse, 2009; Tombaugh, 2004). Along these lines, there are critical degrees of decline, likely accompanied and/or promoted by one or more of the AD biomarkers and genetics described above, that can result in a diagnosis of MCI and ultimately probable AD/senile dementia of the Alzheimer type.

As will be seen below (*in “G. Perceptiv can Improve Diagnostic Classification of Individuals with Alzheimer’s disease”*), some individuals initially performing at a level consistent with moderate AD improved following receiving the Perceptiv formulation to the extent that they would be classified as having mild AD, and some of those initially performing at a level consistent with mild AD improved to the extent that they would be classified as cognitively normal. The Perceptiv

formulation improved or maintained executive function (complex cognitive performance) for individuals with no known or suspected cognitive performance, individuals diagnosed with MCI and individuals diagnosed with AD. This was quantified by performance on the same tests (Clock-Drawing Test and DRS) for individuals with MCI and AD and for another executive function test (the Trail-Making Test) for individuals with no known or suspected cognitive difficulties. Note that the Clock-Drawing Test is not an appropriate executive function test instrument for individuals with no known or suspected cognitive difficulties, as they will display a perfect or near-perfect score (as was seen for “intact” individuals diagnosed with MCI; Fig. 6; derived from Shea and Remington, 2019); executive function for individuals with no known or suspected cognitive difficulties was assayed using the Trail-Making Test, which is time-sensitive and therefore has no inherent maximum score. Nevertheless, both the Clock-Drawing and Trail-Making Tests quantify executive function.

This is compelling evidence that the formulation benefited individuals diagnosed with AD by the same mechanism as it benefitted individuals with MCI and individuals with no known or suspected cognitive difficulties. This is corroborated by the persistent association of the most prominent AD biomarker (Abeta) and the highest genetic risk factor (the ApoE4 genotype) with relatively impaired cognitive performance among individuals still performing within a cognitively normal range, coupled with clinical studies demonstrating mitigation of onset and progression of AD by one or more components of the Perceptiv formulation as described above.

In this regard, the *FDA Guidance for Industry: Evidence-Based Review System for the Scientific Evaluation of Health Claims* states:

“FDA considers evidence from studies with subjects who have the disease that is the subject of the claim only if it is scientifically appropriate to extrapolate to individuals who do not have the disease. That is, the available scientific evidence demonstrates that (1) the mechanism(s) for the mitigation or treatment effects measured in the diseased populations are the same as the mechanism(s) for risk reduction effects in non-diseased populations and (2) the substance affects these mechanisms in the same way in both diseased and healthy people.¹¹”

Improvement in cognitive performance for individuals with AD is evidence that the Perceptiv formulation operates by the same mechanisms for these individuals as it does for individuals with no known or suspected cognitive difficulties and for individuals diagnosed with MCI as seen above. *Consideration of these findings with individuals diagnosed with AD are therefore relevant to the proposed Qualified Health Claim. As such, the next two sections (F and G) demonstrate that the Perceptiv formulation improved cognitive performance to the extent that a prior diagnosis of AD would be altered following consumption of the Perceptiv formulation.*

F. The Perceptiv Formulation is Effective for Multiple Stages of Alzheimer’s Disease

In the above randomized trial for individuals diagnosed with AD, Remington et al. (2015b) also classified participants according to their respective baseline AEMSS-adjusted DRS scores as Cognitively Intact (18-9), Mild Dementia (8-6), Moderate Dementia (5-4) and Severe Dementia (3-0) as defined in the DRS manual (Jurica et al., 2001), and compared the impact of the formulation among these categories. The Perceptiv formulation imparted robust improvement in cognitive performance within 3 months for participants classified as cognitively intact or having

¹¹ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-evidence-based-review-system-scientific-evaluation-health-claims#ftn20>. Last accessed 9/6/2019.

mild and moderate dementia but had less impact on cognitive performance for those participants with severe dementia (Table 8).

Table 8: Relative efficacy of the Perceptiv formulation on cognitive performance for participants with varying stages of Alzheimer's disease.

Diagnosis	Change Perceptiv vs Placebo*
Cognitively Intact	2.5 ± 1.8
Mild Dementia	1.7 ± 0.8
Moderate Dementia	2.0 ± 0.7
Severe Dementia	0.4 ± 0.7

Participants were classified according to their baseline AEMSS scores described within the text. Values represent the mean change in cognitive performance (\pm standard error of the mean) for the cohort randomized to Perceptiv versus the cohort randomized to placebo after 3 months calculated as the (change from baseline for participants randomized to Perceptiv) – (change from baseline for participants randomized to placebo). *Data from Remington et al., 2015b.

This differential efficacy corroborates our earlier studies, in which individuals displaying cognitive performance characteristic of relatively mild/moderate dementia improved statistically after receiving the Perceptiv formulation (Chan et al., 2008a), while individuals displaying performance characteristic of more severe dementia did not improve after receiving the Perceptiv formulation but displayed an attenuated decline versus those receiving placebo (Remington et al., 2009).

G. The Perceptiv Formulation can Improve Diagnostic Classification of Individuals with Alzheimer's disease

Assignment of mean participant performance on the AEMSS-adjusted DRS to the above participants diagnosed AD (Remington et al., 2015b) underscores the extent of improvement in cognitive performance attained following consumption of the Perceptiv formulation. Following randomization, baseline scores for Treated and Placebo cohorts were both within the range classified as "Moderate Dementia" (5-4 points; Jurica et al., 2001) and were statistically identical. After 3 months, the cohort randomized to Perceptiv had a mean score that improved statistically ($p<0.001$) and clinically (Effect Size = 0.24) to 6.7, which not only differed statistically from that of the cohort randomized to placebo ($p<0.05$), but corresponds to a classification of "Mild Dementia" (6-8 points; Jurica et al., 2001). By contrast, the cohort randomized to placebo had a mean score of 5.1, which did not differ statistically or clinically from their baseline performance and retained their original classification as "Moderate Dementia" (Table 9).

Table 9: Impact of Consuming the Perceptiv formulation on Alzheimer Diagnosis

	Baseline ^x	3 months
Perceptiv	4.5 ± 0.5 ^{xx}	6.7 ± 0.6
Diagnostic Classification ^{xxx}	Moderate Dementia	Mild Dementia
Placebo	4.3 ± 0.6	5.1 ± 0.7
Diagnostic Classification	Moderate Dementia	Moderate Dementia

^xData from Remington et al., 2015b

^{xx}Values indicate mean \pm standard error of the mean

^{xxx}Classification according to the DRS manual (Jurica et al., 2001)

As shown above (Table 9), there was a range of baseline performance among individuals in the above cohorts. We next compared the subset of individuals in the cohorts randomized to

Perceptiv and to placebo for whom baseline AEMSS performance classified them as having “Mild Dementia” (6-8; Jurica et al., 2001). Prior to treatment, these cohorts displayed statistically identical performance, but following treatment, the cohort randomized to the Perceptiv formulation displayed statistically improved performance versus the cohort randomized to placebo ($p<0.05$; Table 10). Moreover, after 3 months, the cohort randomized to Perceptiv had improved statistically ($p<0.015$) and clinically (Effect Size = 0.47) versus their own collective baseline, which indicated not only statistical improvement versus the placebo cohort ($p<0.05$) but that they had *improved to the extent that their scores now classified them as “Cognitively Intact.”* By contrast, the cohort receiving placebo did not improve, and their scores continue to classify them as having “Mild Dementia” (Table 10). *Participants were not re-diagnosed; these comparisons are intended to provide a context of the extent of efficacy of Perceptiv on cognitive performance.*

Table 10: Impact of the Perceptiv formulation on Alzheimer Diagnosis

	Baseline ^x	3 months
Perceptiv	7.1 ± 0.9^{xx}	9.6 ± 0.8
Diagnostic Classification ^{xxx}	Mild Dementia	Cognitively Intact
Placebo	6.7 ± 0.9	6.3 ± 1.1
Diagnostic Classification	Mild Dementia	Mild Dementia

^xData from Remington et al., 2015b

^{xx}Values indicate mean \pm standard error of the mean

^{xxx}Classification according to the DRS manual (Jurica et al., 2001)

These findings for individuals diagnosed with AD parallel those described above for individuals with MCI receiving the Perceptiv formulation; individuals demonstrating intact performance on the DRS also did not display improvement in the Clock-Drawing test versus placebo, while those with impaired DRS performance improved statistically versus their own baseline and versus those receiving placebo (Fig. 6; Shea and Remington et al., 2019).

Improvement in cognitive performance for individuals with AD is evidence that the Perceptiv formulation operates by the same mechanisms for these individuals as it does for individuals with no known or suspected cognitive difficulties and for individuals diagnosed with MCI as seen above. Consideration of these findings with individuals diagnosed with AD are therefore relevant to the proposed Qualified Health Claim¹².

H. Clinical Studies with Individual Ingredients of the Perceptiv Formulation Display Efficacy Directly Relevant to Prevention of Multiple AD-Related Pathologies

The ingredients of the Perceptiv formulation have been the subject of several clinical studies either individually, or in combination with other vitamins and supplements, with some degree of efficacy. However, none of the individual ingredients, nor any subset of ingredients, have displayed the impact observed with the complete Perceptiv formulation. This highlights the usefulness of a multi-targeted approach for nutraceuticals as well as pharmacological agents (Cummings et al., 2019a, b; Panza et al., 2009; Shea and Remington, 2012b).

In this regard, a US patent (Appendix F) was awarded based on the clinical impact of the Perceptiv formulation as seen in the above phase studies for adults with no known or suspected

¹² <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-evidence-based-review-system-scientific-evaluation-health-claims#ftn20>. Last accessed 10/19/2019.

cognitive difficulties and for those diagnosed with AD (Chan et al., 2008a; Remington et al., 2009).

Folate with and without other B vitamins (including B12) improved cognitive performance for individuals with MCI and AD in some but not all studies (Aisen et al., 2008; Connelly et al., 2008; Durga et al., 2007; Kang et al., 2008; Ma et al., 2015; Rommer et al., 2016; Sun et al., 2007; Gillete-Guyonnet et al., 2013; Famenini et al., 2017; James et al., 2019). A 6-year observational, retrospective study of plasma analysis and cognitive performance of elderly individuals demonstrated that folate deficiency was associated with deficient performance, and that elevated homocysteine potentiated further cognitive deficiency (Baroni et al., 2019).

B12 improved cognitive performance in older adults in some but not all studies (Feng et al., 2009; Tangney et al., 2009; van Dyck et al., 2009; Gillete-Guyonnet et al., 2013; Famenini et al., 2017; De Wilde et al., 2017; James et al., 2019). Individuals ≥80 years of age with deficient B12 levels were at increased risk of cognitive decline (Da Rosa et al., 2019).

Vitamin E provided cognitive benefit in some but not all studies for individuals diagnosed with AD, which may derive from the inherent difficulties of nutritional clinical trials (Browne et al., 2019; Berman and Brodaty, 2004; Esposito et al., 2002; Isaac et al., 2008; Kang et al., 2006; Lloret et al., 2009; Morris et al., 2002, 2005; Ortega et al., 2002; Petersen et al., 2005; Farina et al., 2017; De Wilde et al., 2017; Gillete-Guyonnet et al., 2013; Famenini et al., 2017). Vitamin E improved activities of daily living for individuals diagnosed with AD and reduced progression of AD (Dysken et al., 2014; Sano et al., 1997). Vitamin E may slow functional decline for individuals diagnosed with AD, which was reflected by a decrease in caregiver burden (Dysken et al., 2014; Farina et al., 2017). Efficacy of vitamin E supplementation was observed in individuals with low baseline intakes, while those already consuming the recommended daily allowances did not show benefit (Kang et al., 2006, 2008).

N-acetyl cysteine demonstrated impact on cognitive performance for individuals with AD (Adair et al., 2001; James et al., 2019) and improved cognitive performance in a single case study for an individual with AD already receiving folic acid and B12 (McCaddon and Davies, 2005).

Acetyl-L-carnitine improved cognitive performance (Montgomery et al., 2003; De Jesus Moreno Moreno, 2003; James et al., 2019), with superior effect on individuals <65yrs of age (Thal et al., 1966, 2000). Acetyl-L-carnitine also improved for cognitive performance and BPSD when administered to individuals diagnosed with AD that were already receiving, but were not responsive to, pharmacological agents (donepezil or rivastigmine; Bianchetti et al., 2003)

S-adenosyl methionine provided limited efficacy for cognitive performance in AD (Rudolph et al., 2011; James et al., 2019; Levkovitz et al., 2011) and for depression (Bottiglieri, 2002; Mischoulon and Fava, 2002; Levkovitz et al., 2011); the latter is relevant since depression can potentiate the conversion of MCI to AD (Mourao et al., 2016; Montegro and Fernandez, 2004), can negatively influence overall outcomes and is associated with the ultimate need for institutionalization (Ortega et al., 2017; Chakraborty et al., 2019; Silva et al., 2019).

The above clinical findings corroborate the efficacy of individual ingredients of Perceptiv, and highlight the superior efficacy derived from the complete Perceptiv formulation.

I. Summary of Substance-Disease Relationship

As articulated more fully in Section IID (above), our clinical studies demonstrate that the Perceptiv formulation (the substance) improves or maintains cognitive performance in adults with

no known or suspected cognitive deficiencies and in individuals diagnosed with MCI, which is contingent upon prevention of progressive damage to, and loss of function of, the brain that can result in AD (the disease), therefore *reducing the risk of AD in the elderly (the appropriate subgroup of the US population)*. Diagnosis following a critical decline in cognitive performance remains the definitive criterion for "senile dementia of the Alzheimer type" or "probable Alzheimer's" pending autopsy. Since the Perceptiv formulation improves or maintains cognitive performance in adults with no known or suspected cognitive difficulties and in individuals diagnosed with MCI, it would by definition take longer for an individual to decline in cognitive performance to the extent that a diagnosis of AD would be warranted. It is moreover possible that an individual consuming the Perceptiv formulation would never decline to that extent during their lifetime; our clinical studies therefore establish a relationship linking the substance and disease. ***These findings establish a Substance-Disease Relationship between the Perceptiv formulation and the risk of AD.***

Our pre-clinical studies identify the Perceptiv formulation as alleviating, reducing, preventing and/or mitigating multiple aspects of neuropathology that underly the development and progression of AD, which translates into reduction of risk of developing AD, including reduction or prevention of establishment of definitive biomarkers (senile plaques) that would allow a diagnosis of AD following autopsy, they further support a relationship linking the substance and disease. Multiple clinical and preclinical studies by others, using one or more components of the Perceptiv formulation, corroborate our findings.

IV. MINIMUM EFFECTIVE DOSE

The minimum effective dose is daily consumption of the concentration of each component as listed above in section "II. PRELIMINARY REQUIREMENTS," subsection "A. Description of the supplement" as follows:

- Folic acid: 400µg
- B12: 6 µg
- Vitamin E: 30 I.U.
- N-acetyl cysteine: 600mg
- Acetyl-L-carnitine: 500mg
- S-adenosylmethionine: 400mg (200mg of which is active ion)

These were the concentrations consumed daily in all clinical studies described herein. In further support of this minimum effective dose, Chan et al. conducted a pilot study (unpublished), in which 12 individuals with no known or suspected cognitive difficulties were randomized to half of the dosage of the Perceptiv formulation or placebo daily for two weeks. Cognitive performance under these conditions was not altered using the same test instruments as in Chan et al., (2010); by contrast, as shown above, participants randomized to the full daily Perceptiv dosage demonstrated cognitive improvement. No further variations of the Perceptiv formulation were tested.

V. NATURE OF FOOD ELIGIBLE TO BEAR THE HEALTH CLAIM

While the ingredients of the Perceptiv formulation are present in various foods and/or derived during normal metabolism, no one food contains all of the ingredients. Accordingly, our Qualified Health Claim petition is confined to the Perceptiv product and does not extend to any foods.

VI. LABELING REQUIREMENTS

A. Novel Labels for Food

N/A since no foods contain the Perceptiv formulation.

B. Supplement Label

The Perceptiv label contains the following description of ingredients:

Supplement Facts		
Serving Size: Two (2) caplets		
	Amount Per Serving	% DV
Vitamin E (Natural D-Alpha-Tocopherol Acetate)	30 IU	100%
Folic Acid	400mcg	100%
Vitamin B12 (Cyancobalamin)	6mcg	100%
Proprietary Blend: N-Acetyl L-Cysteine, Acetyl L-Carnitine, S-Adenosyl Methionine (SAMe)	1500mg	*

*Daily Value not established

The label can be viewed in Appendix G. No changes in description of the ingredients are proposed.

VII. ANALYTICAL DATA

A. Analysis of Food

N/A since no foods contain the entire Perceptiv formulation.

B. Analysis of the Perceptiv product

A representative Certificate of Analysis from **ABH Nature's Products** (the company that manufactures Perceptiv for Sevo Nutraceuticals) is included in Appendix H.

VIII. PROPOSED MODEL QUALIFIED HEALTH CLAIMS

The proposed Qualified Health Claims presented below are based entirely upon clinical studies using the Perceptiv formulation. However, additional support and rationale for the composition of the Perceptiv formulation were derived from clinical studies using one or more ingredients of the Perceptiv formulation. The rationale for including those studies is that many details of metabolism and the complex enzymatic pathway interplay that elucidate the underlying mechanisms could only be worked out using murine models, cell culture and/or cell-free reactions with purified metabolites. This is analogous to our knowledge of the development and progression of AD, which led to the development of pharmacological treatments, while association of AD with certain genetic profiles and biomarkers fostered detailed examination with transgenic mouse models, cell cultures and cell-free studies to elucidate responsible mechanisms.

A. Importance of the Proposed Qualified Health Claim

The current label states that Perceptiv has been "*Clinically proven to enhance mental clarity, help improve memory, and help restore a healthy mood,*" and that Perceptiv "*protects against the normal cognitive decline associated with aging.*" These limited structure-function claims, for which the FDA received appropriate notification in 2012 (Appendix I), were based upon the published, randomized, double-blind clinical trial with adults with no known nor suspected cognitive impairment (Chan et al., 2010, as presented above). No reference to the risk of individuals with no cognitive difficulties, or those individuals diagnosed with MCI, developing AD has been included pending FDA approval of the present petition.

Unfortunately, in omitting such references, neither the label nor general advertising convey the wealth of clinical evidence that (1) the Perceptiv formulation may reduce the risk of development of AD, (2) that the formulation has improved or maintained cognitive performance for individuals independently diagnosed with MCI or AD, and moreover is also effective for management of Behavioral and Psychological Symptoms of Dementia, for periods as long as one year in observational follow-up analyses of intervention studies (Remington et al., 2015a,b, 2016, 2009) and >2 years in one extended observational study (Chan et al., 2008a).

Accordingly, most if not all individuals with normal cognitive performance, medical professionals, caregivers, and individuals themselves diagnosed with MCI, senile dementia of the Alzheimer's type, or probable Alzheimer's disease, yet cognizant enough to be pro-active, are unlikely to perceive Perceptiv as an appropriate part of any preventative or intervention unless they conduct considerable searches of the published medical literature on their own. Even if they do search published literature, many/most will not perceive that Perceptiv (a brand name) is one and the same as the "Nutraceutical Formulation" described in the above publications. Inclusion of Qualified Health Claims conveying the positive impact of Perceptiv at reducing the risk of developing AD is therefore critical to allow such individual to evaluate whether or not to include Perceptiv as part of their regimen to maintain cognitive function and manage behavioral difficulties during progression of their respective conditions.

We consider that amending the label via appropriate Qualified Health Claims as proposed herein is consistent with the spirit intent of the Nutrition Labeling and Education Act of 1990, which sought to prevent unnecessary exclusion of critical disease-related information from food/supplement labels¹³.

B. Proposed Qualified Health Claim for Label

Based upon the published clinical studies with the Perceptiv formulation reviewed herein, we propose the following qualified health claim:

"Clinical studies provide evidence that Perceptiv can reduce the risk of Alzheimer's disease."

and

"Clinical studies provide evidence that Perceptiv can reduce the risk of age-related cognitive decline."

While not a health claim but rather a structure/function claim, we propose to include the statement,

"Clinical studies provide evidence that Perceptiv can improve cognitive performance across the adult life span."

C. Proposed Information to be Added to the Perceptiv Website

Sevo Nutraceuticals maintained a website (www.perceptiv.com) that included extensive information regarding Perceptiv, including downloadable PDFs of the above published clinical studies, and additional important information for consumers. This website is not currently in use but will be restored with modifications pending the conclusion of FDA review of this petition. In accord with the above Qualified Health Claims, we propose to add the following information in a

¹³<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm056975.htm#foot1> last accessed January 4, 2016

prominent location at this website:

"As described on the Alzheimer's Association website (<http://www.alz.org/what-is-dementia.asp>), "Dementia is a general term for a decline in mental ability severe enough to interfere with daily life. Memory loss is an example. Alzheimer's disease is the most common type of dementia. Dementia is not a specific disease. It is an overall term that describes a wide range of symptoms associated with a decline in memory or other thinking skills severe enough to reduce a person's ability to perform everyday activities. Alzheimer's disease accounts for 60 to 80 percent of cases. Vascular dementia, which may occur after a stroke, is the second most common dementia type. But there are many other conditions that can cause symptoms resembling dementia, including some that are reversible, such as thyroid problems and vitamin deficiencies.

Perceptiv® is *not* a treatment for dementia, Alzheimer's disease, Mild Cognitive Impairment, nor any other cognitive disease, but clinical studies indicate that Perceptiv® can reduce the risk of developing Alzheimer's disease. Perceptiv® is not a substitute for any drug or product recommended by your physician. Consult your physician before taking Perceptiv® or making any other changes to your daily regimen."

D. Public Health Benefit That Will Derive from Use of the Claim (included in Appendix J)

Progression of AD, and resultant decline in ability to carry out daily activities, requires progressively increasing care. As stated in the Introduction, health care expenses are expected to increase to \$1.1 trillion by 2050 (Stefannaci, 2011). The impact of AD therefore extends beyond that of affected individuals and their caregivers and places a tremendous burden on our nation's health care system (Alzheimer's Association Report, 2016). *As shown above, Perceptiv improved cognitive performance for individuals with normal cognitive performance and delayed and/or reversed cognitive and behavioral decline for individuals diagnosed with MCI and AD; the public health benefit that can be derived from allowance of the proposed Qualified Health Claim cannot be underestimated.*

E. Who Will Benefit by the Addition of the Proposed Claims (included in Appendix J)

Since AD is an age-related disorder, the Target Group that will directly benefit by the proposed health claims are the elderly individuals of the General Population. Factors in support of this follow:

- (1) All surviving individuals in the General Population will eventually reach the age range of at which they are at risk of developing AD or MCI.
- (2) The number of people with AD doubles every 5 years beyond age 65. Approximately 30% of people age 85 and older in the US have AD¹⁴
- (3) Approximately 200,000 individuals have early-onset AD (i.e., <65 years of age).
- (4) AD affects all races. However, a significantly increased prevalence of Alzheimer's disease has been noted in elderly African-Americans and Hispanics as compared to elderly Caucasians in the United States (Alzheimer Association Report, 2016).

¹⁴ <https://www.alz.org/alzheimers-dementia/what-is-alzheimers/causes-and-risk-factors>. Last accessed 10/20/2019.

(5) 40-50% of individuals diagnosed with MCI will convert to AD (Langa and Levine, 2014; Croisile et al., 2012). However, it has been considered that conversion is not inevitable (Bruscoli and Lovestone, 2004), supporting pro-active intervention.

(6) AD has multiple genetic causes, indicating that it can be passed on through families¹⁵. While most genetic causes are recessive, some are dominant, indicating that there is a 50% chance of inheritance.

(7) Nutrition declines in general with age and declines even more critically in AD; nutritional deficiencies potentiate AD (Shatenstein et al., 2007 and additional references above).

(8) The impact of AD also extends to caregivers. Even the early stages of cognitive decline promote caregiver stress (Krolak-Salmon et al., 2019). As AD becomes progressively more severe for individuals, their respective caregivers undergo anxiety, depression, and extreme grief, all of which contribute to overall decline in caregiver health (Cooper et al., 2007; Sanders et al., 2008; Garcia-Alberca et al., 2012; Raggi et al., 2015; Schulz and Martire, 2004; Gaugler et al., 2009; Noyes et al., 2010).

(9) The continuum between normal cognitive performance and a decline sufficient to warrant a diagnosis of AD is underscored by the demonstration that, as described above, (1) multiple aspects of cognitive decline directly related to AD begin during young adult years and continue throughout life, (2) Alzheimer-specific biomarkers can precede critical cognitive decline by 10 years or more (3) genotypes increasing the risk for AD are by definition present throughout life and (4) epigenetic risk factors increase during aging.

These data underscore that the General Population is at risk for developing AD, and all individuals in the General Population as they age can therefore be considered to benefit directly from the proposed Qualified Health Claims.

F. Safety of the Perceptiv Formulation (included in Appendix K)

No serious adverse events were reported in the above clinical studies with the Perceptiv formulation. The formulation was well-tolerated: >394 participants consumed the formulation daily for periods ranging from 2 weeks to 2.4 years in our studies with no serious adverse events. Of these participants, 324 consumed the formulation daily for 3 months, 180 consumed the formulation daily for 6 months, 73 for 9 months, and 49 for ≥1 year, respectively (Chan et al., 2008a, 2010; Remington et al., 2009, 2015a, b, 2016).

Prior reports (Carney et al., 1989) indicated that consuming greater than 800mg of S-adenosylmethionine may cause increased anxiety and mania in patients affected with bipolar depression. The Perceptiv formulation contains only half of this concentration (400mg) as a total daily dose. While Carney et al. (1989) reported that no deleterious effects were observed following consumption of less than 800mg, bipolar depression was nevertheless an exclusion criterion in our clinical studies (Chan et al., 2008a, 2010; Remington et al., 2009, 2015a,b, 2016) and has been an exclusion criterion in other clinical studies (Rudolph et al., 2011). The Perceptiv label includes, and will continue to include, the recommendation that individuals with bipolar disorder should consult their physician before consumption, despite that the Perceptiv formulation contains a total daily dose of only 400mg.

¹⁵ <https://www.nia.nih.gov/alzheimers/publication/alzheimers-disease-genetics-fact-sheet>. Last accessed 10/20/2019.

Even when administered intravenously (which maximizes absorption) in doses up to 500mg, S-adenosylmethionine was eliminated with a half-life of approximately 90 min and near complete elimination within 24hr (Gulidori et al., 1984). Further in this regard, individuals with AD display low levels of S-adenosyl methionine (Kennedy et al., 2004; Bottigieri et al., 1990) making it even less likely that such individuals would approach the above cautionary level following consumption of the Perceptiv formulation.

IX. ENVIRONMENTAL IMPACT STATEMENT

We request a categorical exclusion from providing an Environmental Impact statement under 21C.F.R. §25.32¹⁶. Accordingly, an environmental impact statement is not required.

X. LIST OF APPENDICES

- Appendix A: Background of the Petitioner
- Appendix B: FDA IND Number for the Perceptiv formulation
- Appendix C: Demographics and Performance in Trail-Making and Digit-Recall Test
- Appendix D: Comparison of Treated and Placebo Cohorts from Remington et al. (2015a).
- Appendix E: Clinical Studies with the Perceptiv Formulation: Studies That Directly Evaluate the Substance/Disease Relationship
- Appendix F: US Patent for the Perceptiv formulation
- Appendix G: Prior label of the Perceptiv product
- Appendix H: Analysis of the Perceptiv Product
- Appendix I: Notification to FDA of Original Structure-Function Claims
- Appendix J: Information Pertaining to the U.S. Population (Taken from main document)
- Appendix K: Safety of the Perceptiv Formulation
- Appendix L: References
- Appendix M: Computer searches

Yours very truly,



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¹⁶ http://www.ecfr.gov/cgi-bin/text-idx?SID=7a654e4b78e97bdb391c861009c57c8&mc=true&node=pt21.1.25&rgn=div5#se21.1.25_132;
Last accessed 10/20/2019.

Appendix D: Comparison of Treated and Placebo Cohorts from Remington et al., 2015a

Clock-Drawing Test			
Treated		Placebo	
Baseline	6 months	Baseline	6 months
12	8	15	15
14	15	12	15
14	15	15	15
14	15	12	13
14	14	15	13
14	15	8	3
15	15	15	12
	14	14	8
14	12	9	
13	13	15	13
11	11	12	10
14	15	15	14
13			
15			
15	15		
14			
10	14		
14	15		
14	12		
14	15		
14	14		
12			

	p value
Treated vs. Placebo	0.045
Treated vs. Baseline	0.351
Placebo vs. Baseline	0.038

Values represent participant scores on the Clock-Drawing tests at baseline and after 6 months as indicated. *P* values were derived by 1-tailed Student's t test. "Treated vs. Placebo" indicates comparison of values at 6 months. "Treated vs. Baseline" indicates comparison of values at 6 months versus respective values at Baseline, and "Placebo vs. Baseline" indicates comparison of values at 6 months versus respective values at Baseline. Note the significant difference between Treated vs. Placebo cohorts, maintenance of baseline performance of the Treated cohort, and significant decline after 3 months versus baseline performance of the Placebo cohort.