Title:	Photobiomodulation for Improving Brain Function in Dementia (PBM Dementia)
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Examining the Impact of Photobiomodulation (PBM) on Brain Function in Dementia – Protocol

Protocol Summary

Study Objectives:	1. Replicate the findings of Saltmarche et al. (2017) on the effects of PBM treatment on cognitive function in older adults with dementia. 2. Examine whether 12 weeks of PBM therapy with the Vielight Neuro device improves default mode network (DMN) functional connectivity and cerebral blood flow (CBF) in older adults with dementia. 3. Examine the effects of PBM treatment on quality of life in older adults with dementia and on caregiver burden.
Study Design:	Randomized, wait-list controlled trial
Study Duration:	25 weeks
Number of Study Subjects:	10 patients with dementia and their primary caregivers
Main Inclusion Criteria:	<u>Primary Study Participants (PP):</u> • Diagnosis of dementia, preferably Alzheimer's dementia • 50 years of age or older • English language fluency • No contraindications for MRI • Has legally authorized representative (LAR) consent <u>Caregivers (CG):</u> • Currently provides care for PP • Ability to answer questions about PP's behaviors, quality of life, and own stress levels
Summary of Study Procedures:	Ten pairs of older adults with dementia and their caregivers will be enrolled in a randomized, wait-list control trial. Five primary participants, PP, (i.e., individuals with dementia) will be randomized to start PBM treatment immediately with the Vielight Neuro device after the baseline psychometric and MRI assessments. Five PP will be randomized to a "Delayed PMB" group that will not start treatment with the Vielight Neuro device until after the 12-week psychometric and MRI assessment. Cognitive function and quality of life will be assessed in all PPs at baseline, weeks 6, 12, 18, and 24. Caregiver burden, positive aspects of caregiving, depressive symptomology, and dementia-related behaviors in the PP will be assessed in all caregivers (CG) at baseline, weeks 6, 12, 18, and 24. Neuroimaging measures will only be assessed in PPs at baseline and week 12.
Study intervention:	The experimental intervention will be PBM treatment with the Vielight Neuro device. The device uses clusters of 3 light emitting diodes (810nm wavelength). Diodes are placed on the skull at equidistance as well as intranasally to target different brain areas relevant to dementia. No significant heat is generated. PPs will be treated in 20 min sessions once daily, 5 days/week for at least 12 weeks. CG will be taught to use the device at home and maintain a treatment log/diary.
Study Aims:	1) Replicate Saltmarche et al. (2017)'s finding that 12 weeks of PBM

	therapy improves cognitive function in patients with dementia. 2. Examine the effect of 12 weeks of PBM therapy on DMN functional connectivity and cerebral blood flow in individuals with dementia. 3. Examine the effects of 12 weeks of PBM therapy on quality of life in individuals with dementia and caregiver burden. 4. Determine if changes in cognition and quality of life are associated with changes in DMN functional connectivity and cerebral perfusion after 12 weeks of PBM therapy.
Study Hypotheses:	1) Primary participants (PP) randomized to in the Immediate PBM group will show improvements in cognitive function after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared to PP randomized to the Delayed PBM group. 2) PP randomized to the Immediate PBM group will show increased functional connectivity within nodes of the DMN after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared to PP randomized to the Delayed PBM group. 3) PP randomized to Immediate PBM will show increased cerebral perfusion after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared PP randomized to the Delayed PBM group. 4) Improvements in cognitive function (Alzheimer's Disease Assessment Scale-cognitive subscale, ADAS-cog, and the Montreal Cognitive Assessment, MOCA) and Quality of Life in Alzheimer's Disease (QOL-AD) scores will be correlated with increases in DMN functional connectivity and increases in cerebral perfusion after 12 weeks of PBM therapy with the Vielight Neuro in PP randomized to Immediate PBM 5) Caregivers in the Immediate PBM group will experience greater reductions in caregiver burden (Caregiver Burden Inventory, CBI) and depressive symptomology and improvements in positive feelings about caregiving (Positive Aspects of Caregiving) after 12 weeks compared to caregivers in the Delayed PBM group.
Statistical Methodology:	We will use a two sample t-test on the baseline vs. week-12 differences in PP cognitive measures to examine between-group differences to test hypothesis 1. A functional connectivity analysis produced coefficients from a priori defined seed-voxel correlation with Pearson correlation analysis will be used. Fisher's r-to-z transformation will be used to convert correlation maps into z maps. Random effect models will be applied for second level analysis. A two sample t-test on the baseline vs. week-12 differences between the Immediate PBM and Delayed PBM groups (Immediate [baseline-week 12] vs. Delayed [baseline-week 12]) will be used to examine the difference between the two groups for hypothesis 2. We will extract the average cerebral blood flow (CBF) values for each subject by averaging the CBF values within a priori regions of interest (ROIs). Next, we will use a two sample t-test on the baseline vs. week-12 differences between the Immediate PBM and Delayed PBM groups

	(Immediate [baseline-week 12] vs. Delayed [baseline-week 12]) to examine between-group differences for hypothesis 2. We will use Spearman's rank correlation analysis to examine the relationship between baseline-to-week 12 changes in DMN functional connectivity and CBF with baseline-to-week 12 changes in ADAS-cog, MOCA, and QOL-AD in the Immediate PBM group to test hypothesis 3. We will use a two sample t-test on the baseline vs. week-12 differences in CG measures (i.e., Caregiver Burden Inventory, Positive Aspects of Caregiving Scale, Geriatric Depression Scale) to examine between-group differences to test hypothesis 5. We will also evaluate the magnitude of treatment effects on the PP and CG psychometric measures using Cohen d effect sizes for the within-group difference in the Delayed PBM group (baseline-to-week 12 and week 12-to-week 24).

BACKGROUND

I. Dementia and Alzheimer's Disease

Dementia is a global health problem of epidemic proportion with no present cure. It is estimated to affect 5.4 million people in the United States (Alzheimer's World Report, 2016) and 46.8 million people worldwide (World Alzheimer Report, 2016). This represents 1 in 9 individuals aged 65 years or older and 1 in 3 aged 85 or older (Alzheimer's World Report, 2016). Over the next 40 years, it is expected that longer life expectancies coupled with demographic trends will result in a dramatic increase in dementia prevalence, with 16 million cases in the U.S. and 131.5 million worldwide. Alzheimer's disease (AD), the most common cause of dementia in the elderly (Blennow et al., 2006), exacts a deleterious effect on patients, caregivers, and families (Reitz et al., 2011).

Though a few Federal Food and Drug Administration (FDA)-approved pharmacotherapies do exist for the treatment of AD, these produce symptomatic improvements but are not curative nor have been demonstrated to slow the progression of the disease. Thus family members of AD patients are faced with the eventual decision of becoming at-home caregivers or relying on long-term care facilities. Home care assistance can help to lengthen the time AD patients are able to remain living in the community, yet a significant number of patients eventually require long-term care facilities. Severe declines in cognition also result in increased accidents and emergency department visits, often resulting in long-term hospitalization.

Dementia is tremendously costly physically, emotionally, and financially. Affected individuals experience a progressive decline in cognition and physical function that leads to a loss of ability to perform basic daily activities such as dressing, eating, and using the toilet. These functional changes are often associated with emotional strain in caregivers and feelings of stress and burden. In many cases, family caregivers are unable to provide care as the disease progresses, and affected individuals are transitioned to long-term care facilities. In fact, the main component of dementia costs is institutional and home-based long-term care rather than medical services. In the U.S. alone, the cost of caring for individuals with dementia were estimated to be $159 billion to $215 billion in 2010, with a projected increase to $379-$511 billion in 2040 (Hurd et al., 2013). Although home care and long-term care are the largest direct costs of dementia, family caregivers also provide millions of hours of care each year and this is only expected to increase in the coming years. Therefore, an effective treatment for dementia and AD would not only improve memory, cognition and quality of life, but would also have enormous social and economic impact.

II. Currently Available Dementia Treatments

All legally marketed treatments for AD are pharmacological. There are currently 3 pharmacological ligands available with FDA-approved indications for use as treatments of moderate to severe dementia of the Alzheimer's type: donepezil (23mg QD, PO), rivastigmine (13.3mg QD, transdermal patch), and memantine (10 mg BID or 28mg/day QD extended release, PO). Additionally, a combination of donepezil and memantine is available as a single capsule formulation named Namzaric.

Two of these ligands, donepezil and rivastigmine, have their mechanisms of action through non-competitive inhibition of cholinesterases, the enzymes responsible for the hydrolysis of choline esters (Deardorff, Feen et al. 2015). Donepezil is a reversible inhibitor of acetylcholinesterase, the enzyme predominantly responsible for the breakdown of the neurotransmitter acetylcholine, while rivastigmine is a pseudo-irreversible inhibitor of acetylcholinesterase and butylcholinesterase, which plays a minimal role centrally compared to acetylcholinesterase. The neurotransmitter acetylcholine is well-established to play a critical role in learning and memory (Hasselmo 2006). Several deficits in the cholinergic system

have been identified in post-mortem brain examinations of AD (Ferreira-Vieira, Guimaraes et al. 2016), and these ligands are capable of reducing the cognitive and functional deficits experienced by moderate to severe AD patients (Farlow, Salloway et al. 2010; Farlow, Grossberg et al. 2013).

The third ligand, memantine, has its mechanism of action through the anti-competitive antagonism of glutamatergic N-methyl-D-aspartate (NMDA) receptors (Chen and Lipton 1997) across the brain as well as having neuroprotective effects (Hardingham and Bading 2010). Memantine has also been demonstrated to reduce cognitive and functional deficits experienced by moderate to severe AD patients, even when already receiving donepezil (Reisberg, Doody et al. 2003; Tariot, Farlow et al. 2004; Grossberg, Manes et al. 2013).

All of these ligands have their primary mechanisms of action through influences on neurotransmitter systems with evidence of disruption in AD; however, evidence also exists suggesting these ligands affect the mitochondrial dysfunction observed in AD as well. Repeated doses of donepezil or memantine reduce neurotoxic effects and memory impairments caused by okadaic acid (Kamat, Tota et al. 2011) while repeated doses of rivastigamine reduces the biochemical alterations and memory impairments caused by 3-nitropropionic acid in rats (Kumar and Kumar 2009). These pharmacotherapies were demonstrated to protect against those memory impairments due to their ability to preserve mitochondrial function. Thus interest in the mitochondrial cascade hypothesis of AD has been growing, along with evidence of amyloid beta and tau being tied to mitochondrial dysfunction (Spuch, Ortolano et al. 2012; Swerdlow, Burns et al. 2014; Benek, Aitken et al. 2015).

III. Limitations of Currently Available Dementia Treatments

Though several compounds are under clinical investigation for delaying or preventing the progression from the stage of mild cognitive impairment to AD, there have been no successful trials for over 15 years. All FDA-approved therapies for the treatment of AD produce symptomatic improvement only. Though the pharmacotherapies described above are highly valuable in this patient population, there remains a significant need for treatments for long term symptomatic improvement and disease-modifying potential, particularly therapies that can be used in combination with current pharmacotherapeutic options.

Finally, the number of patients experiencing adverse events is considerable, particularly with the cholinesterase inhibitors. In the pivotal trial of 23mg/day donepezil for moderate to severe AD, 74% of patients experienced at least one adverse reaction to the medication with 19% of patients discontinuing the medication for this reason (Farlow, Salloway et al. 2010). The most common adverse reactions included nausea (12%), vomiting (9%), diarrhea (8%), anorexia (5%), and dizziness (5%). In the pivotal trial of 13.3mg/day rivastigmine for severe AD, 75% of patients experienced at least one adverse reaction to the medication with 26% of patients discontinuing the medication for this reason (Farlow, Grossberg et al. 2013). The most common adverse reactions included erythema at application site (13%), agitation (12%), urinary tract infection (8%), dermatitis at application site (8%), and falls (8%). Thus there exists a large proportion of the moderate to severe AD population for whom acetylcholinesterase inhibitors are not an option, as can be seen in the high discontinuation rates (19% and 26%) due to side effects in the two clinical trials.

IV. Vielight Neuro Device

Vielight Inc. has developed a novel photobiomodulation device, the Vielight Neuro. The devices are not labeled for treating dementia or AD and would be described as non-regulated, “low risk general wellness

products," according to the Food and Drug Administration (FDA) document, "General Wellness: Policy for Low Risk Devices", released on July 29, 2016.

Vielight Neuro delivers painless, non-invasive, non-thermal, non-laser, pulsed (40 Hz; 50% duty cycle), near-infrared light (810 nm wavelength) through 5 non-laser light emitting diodes (LEDs) over a 20 minute treatment session. The device is intended for daily use at home and is powered by rechargeable NiMH batteries. One of the Vielight Neuro's LEDs is placed inside the nose for intranasal transmission of light energy and the remainder four LEDs are positioned over the skull for transcranial transmission. The transcranial LEDs are held in position by two lateral stainless steel bands (see Figure 1).

Figure 1. Vielight Neuro device

V. Vielight Neuro's Mechanism of Action

The therapeutic use of near-infrared energy, as delivered by the Vielight Neuro device, is often termed, low level light therapy (LLLT). It uses non-laser LEDs with a near infrared (NIR) wavelength of 810 nm. LLLT has been studied with regards to its effects on a variety of tissue types and clinical indications (Chung, Dai et al. 2012). Product codes have been established for a number of LLLT-based FDA-approved devices, including those for orthodontic treatment (PLH), lymphedema reduction (NZY), pain relief (NHN), hair growth (OAP), wrinkle reduction (OHS), herpes simplex virus cold sore healing (OKJ), fat reduction (OLI), acne treatment (OLP), and fungal infections of the nails (PDZ).

The putative mechanism of action underlying LLLT is termed photobiomodulation (PBM) which is the actions of photonic energy on cellular chromophores. Though the most well-known chromophore is chlorophyll in plants, mitochondrial cytochrome C oxidase (CCO) has also been demonstrated to absorb energy in the NIR range (Karu 1989; Karu and Kolyakov 2005). Also known as complex IV of the electron transport chain, CCO is a critical transmembrane protein with two copper centers and two heme groups, which are likely responsible for the NIR energy absorption. CCO is responsible for reducing cytochrome c and generating water while transporting 4 protons into the intermembrane space.

The involvement of CCO in the pathology of AD has been established through multiple studies (Salminen, Haapasalo et al. 2015; Onyango, Dennis et al. 2016). A post mortem brain examination of AD patients vs. healthy controls found a selective deficit of CCO activity in the temporal cortex and hippocampus of AD patients, but no differences in activity of the other mitochondrial respiratory chain enzyme complexes (Maurer, Zierz et al. 2000). Findings of decreased CCO activity in relevant regions of post mortem brains of AD patients has been observed by several different groups (Kish, Bergeron et al. 1992; Mutisya, Bowling et al. 1994; Parker, Parks et al. 1994; Bosetti, Brizzi et al. 2002). Neurons containing neurofibrillary tangles (Nagy, Esiri et al. 1999) or those composing senile plaques (Perez-Gracia, Torrejon-Escribano et al. 2008) from the post mortem brains of AD patients have both been demonstrated to have low CCO activity. Studies of animal models of AD also confirm that mitochondria in the vicinity of amyloid beta plaques (Xie, Guan et al. 2013), amyloid beta fragments (Canevari, Clark et al. 1999), or even amyloid beta precursor protein (Anandatheerthavarada, Biswas et al. 2003), demonstrate decreased CCO activity and evidence that this decrease occurs prior to the appearance of plaques (Manczak, Anekonda et al. 2006).

The absorption of NIR energy by CCO is believed to cause it to dissociate from nitric oxide (Karu, Pyatibrat et al. 2005; Lane 2006) which is an inhibitor of CCO and causes reduced overall ATP production and can even lead to cell death (Brown 2001). Both amyloid beta and amyloid beta precursor protein have been demonstrated in preclinical models to be capable of concurrently increasing nitric oxide levels, decreasing CCO activity and reducing ATP levels (Keil, Bonert et al. 2004; Keil, Bonert et al. 2004) while the reduction of brain amyloid beta levels by gelosin causes the opposite effects and inhibits apoptosis (Antequera, Vargas et al. 2009). Additionally, upregulation of nitric oxide synthase genes has been observed to correlate with disease severity in post mortem brains of AD patients (de la Monte and Wands 2006).

The resulting effects of LLLT are increased CCO activity (Wong-Riley, Liang et al. 2005), increased electron transport (Pastore, Greco et al. 1994), increased ATP production (Passarella, Casamassima et al. 1984; Karu, Pyatibrat et al. 1995), increased mitochondrial RNA and protein synthesis (Greco, Guida et al. 1989) as well as the modulation of reactive oxygen species leading to activation of nuclear factor kappa B (Chen, Arany et al. 2011), well established as crucially involved in hippocampal synaptic plasticity and memory (Albensi and Mattson 2000; Meffert, Chang et al. 2003). With evidence growing for the hypothesis that mitochondrial dysfunction triggers amyloid beta accumulation (Swerdlow, Burns et al. 2014; Benek, Aitken et al. 2015), it is not surprising that transcranial LLLT has been demonstrated to reduce hyperphosphorylated tau, neurofibrillary tangles, amyloid beta plaques, fragments and precursor protein, while concurrently increasing mitochondrial function, restoring CCO activity and increasing ATP levels in several different transgenic mouse models of AD (De Taboada, Yu et al. 2011; Grillo, Duggett et al. 2013; Purushothuman, Johnstone et al. 2014; Purushothuman, Johnstone et al. 2015).

The Vielight Neuro delivers a synchronized pulse frequency of 40 Hz from all LED clusters. It had been observed that aberrant increases in network excitability and compensatory inhibitory mechanisms in the hippocampus may contribute to Aβ-induced neurological deficits in mouse models (Palop et al. 2007). Electroencephalographic (EEG) recordings in mouse models indicated network hypersynchrony, primarily during reduced gamma oscillatory activity. Restoring gamma oscillatory may inhibit synaptic activity and gamma oscillations and reduced hypersynchrony, memory deficits, and premature mortality – conditions associated with AD (Verret et al. 2014).The gamma pulse frequency of 40 Hz has been demonstrated to attenuate amyloid beta proteins production in the hippocampus and modulate microglial activity resulting in increased scavenging of amyloid beta (Iaccarino, Singer et al. 2016) which may lead to improving AD conditions.

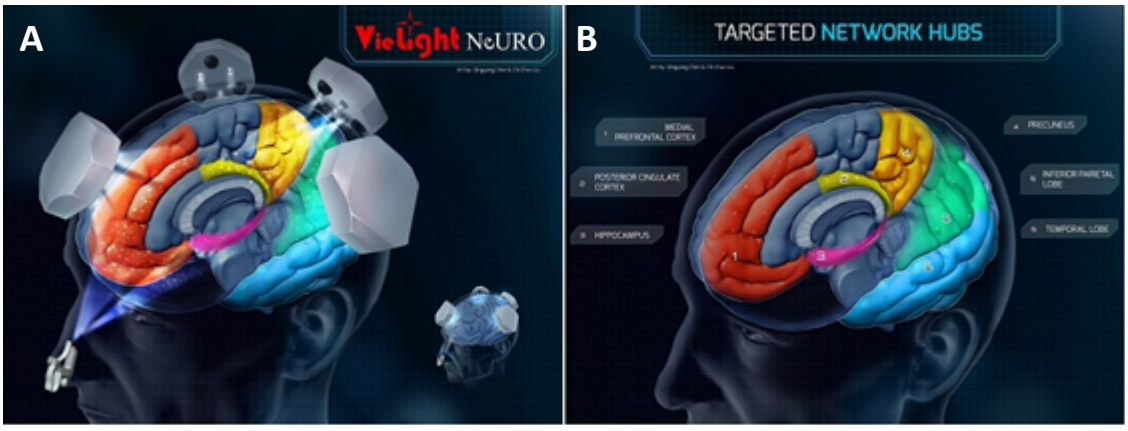


Figure 1. Schematic of how the Vielight Neuro device targets the hubs of the Default Mode Network (DMN).

Finally, the Vielight Neuro has been designed to target the delivery of NIR energy to particular brain regions which are dysfunctional in AD patients, specifically the default mode network (DMN; see Figure 2A). The DMN is a set of brain regions that are concurrently active when an individual is left undisturbed to think to themselves (Shulman, Fiez et al. 1997; Mazoyer, Zago et al. 2001; Raichle, MacLeod et al. 2001). The DMN is also active when an individual is remembering the past, envisioning the future, and considering the thoughts and perspectives of others (Buckner and Carroll 2007). The core regions comprising the DMN (Figure 2B) include the medial prefrontal cortex (mPFC), the posterior cingulate/retrosplenial cortex (PCC/Rsp), the inferior parietal lobule (IPL), lateral temporal cortex (LTC), and hippocampal formation including the entorhinal cortex (Buckner, Andrews-Hanna et al. 2008). AD patients show a reduction in resting glucose metabolism and eventual atrophy in these DMN regions which progresses with disease severity (Minoshima, Giordani et al. 1997; Herholz, Salmon et al. 2002; Scahill, Schott et al. 2002; Thompson, Hayashi et al. 2003). Amyloid beta plaques have also been observed to selectively accumulate in DMN regions, even prior to the observation of symptoms (Klunk, Engler et al. 2004).

VI. Pilot Clinical Data

Saltmarche et al. (2017) conducted an early pilot study using a prior version of the Vielight Neuro technology in 5 patients with mild-to-severe dementia. Inclusion criteria into the study were broad because the study was intended to identify potentially promising patient subpopulations. Baseline characteristics of the patients with dementia are summarized in Table 1.

Table 1. Demographic, and Baseline MMSE and ADAS-cog Scores of the 5 patients with dementia

Patient No.	Baseline MMSE	Baseline ADAS-cog	Age at entry	Sex	Education (yrs)	Dementia Dx (yrs)	dementia medication
1	10	58	77	F	7	2	None
2	10	58	90	M	10+	2	Donepezil
3	21	26.3	76	M	16	0.5	None
4	22	20.7	62	M	10	3.5	Donepezil
5	24	14.2	73	M	18	8	Donepezil
Mean (SD)	**17.4 (6.8)**	**35.5 (21.0)**	**77.6 (7.2)**		**12.2 (4.6)**	**3.2 (2.9)**	

Adapted from Saltmarche et al. (*in press*)

The treatment protocol in pilot study entailed 12-weeks of treatment with the Vielight device followed by a 4-Week, No-Treatment period. During the 12-weeks of treatments, patients went to a clinic to receive a combination of a combined transcranial and intranasal PBM device. In addition, patients received daily treatments at home with an intranasal-only PBM device.

After 12 weeks of PBM, there were significant improvements on the MMSE ($p<0.003$, see **Figure 3**) and the ADAS-cog ($p<0.023$. see **Figure 4**). Increased function, better sleep, fewer angry outbursts, decreased anxiety and wandering was also reported after 12 weeks of PBM treatment. Although there were no negative side effects, there were precipitous declines in cognitive function (e.g., MMSE and ADAS-cog) during the 4-week period of follow-up without treatment.

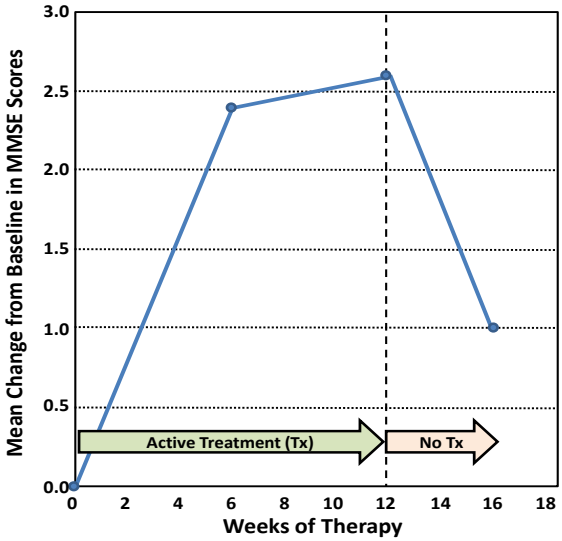


Figure 3. Significant improve on MMSE scores after 12 weeks of PBM treatment with the Vielight Neuro device

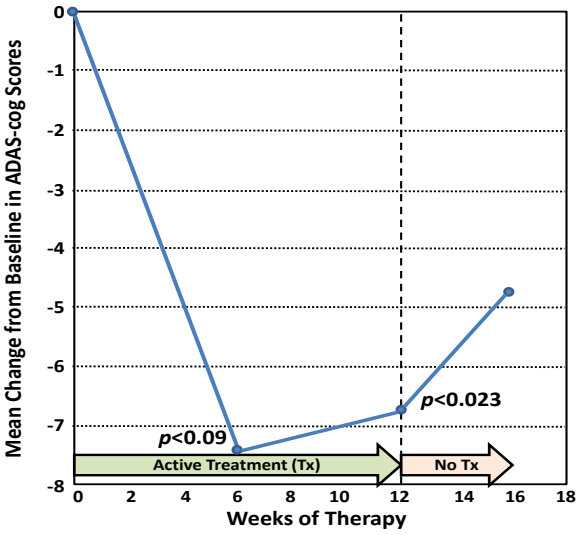


Figure 4. Significant improve on ADAS-cog scores after 12 weeks of PBM treatment with the Vielight Neuro device.

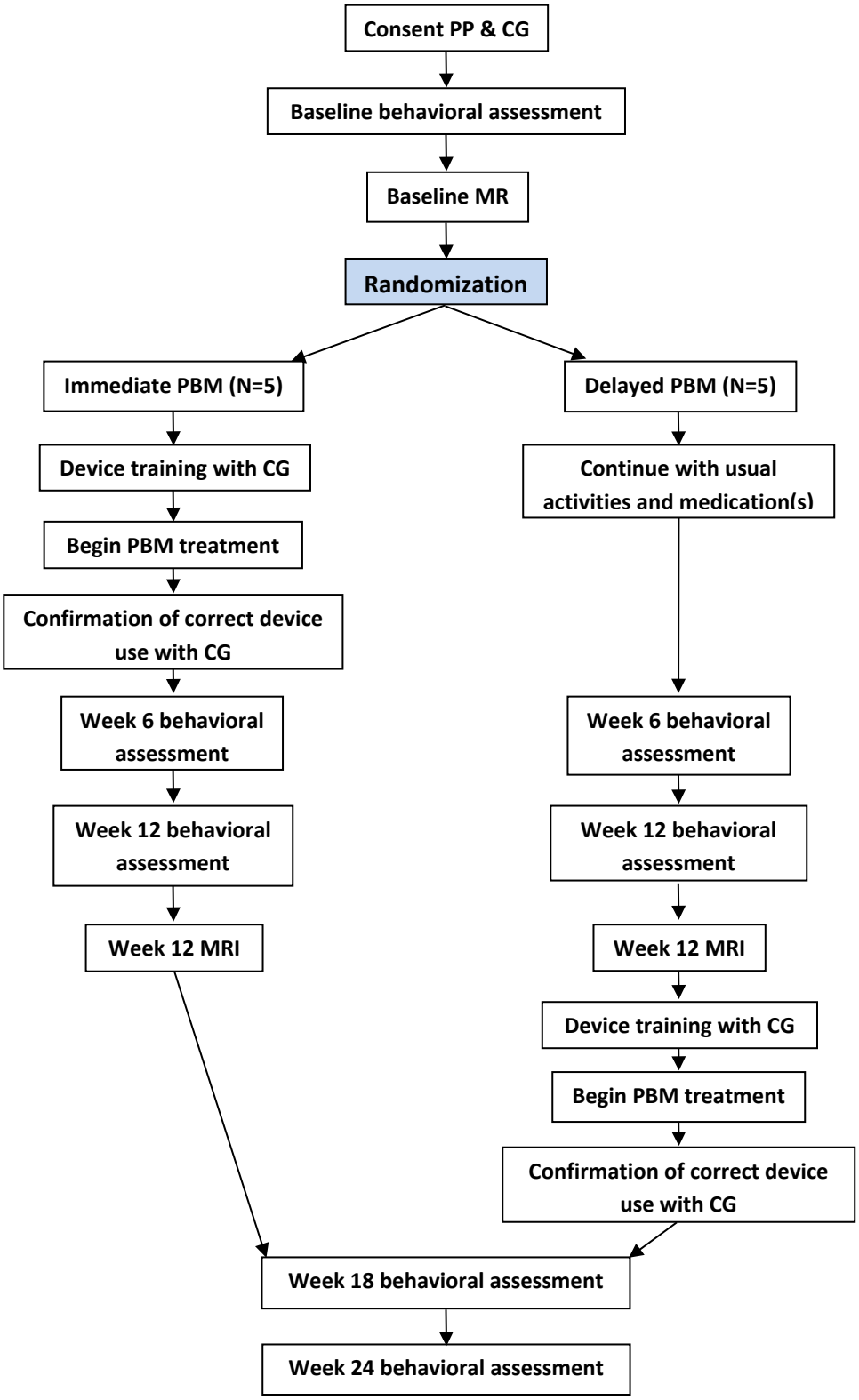
VII. Study Overview:

Photobiomodulation (PBM) is a kind of light therapy that uses red or near-infrared light to stimulate, heal, regenerate, and protect tissue that has either been injured, is degenerating, or else is at risk of dying. Human cadaver studies have shown near-infrared wavelengths can penetrate 40-50 mm through the scalp and skull. Saltmarche et al. (2017) recently showed that 12-weeks of transcranial treatment with a commercially available PBM device (i.e., the Vielight “Neuro") benefits cognitive function in patients with mild-to-moderately severe dementia. Specifically, Saltmarche et al. (2017) reported improvements on the Mini Mental State Examination (MMSE, $p< 0.003$) and Alzheimer’s Disease Assessment Scale-cognitive subscale (ADAS-cog, $p< 0.03$) in 5 dementia patients after 12-weeks of PBM treatment. Because it is important to show that these promising preliminary findings are not spurious findings, the first objective of this study is to replicate Saltmarche et al’s findings that the effects of 12-weeks of PBM treatment can improve cognitive function in older adults with dementia. Because the Vielight Neuro device targets nodes of the default mode network (DMN), which is dysregulated in dementia (e.g., Alzheimer’s disease (AD)), second aim of this pilot study is to investigate whether 12 weeks of PBM therapy with the Vielight Neuro device improves DMN connectivity in older adults with dementia. Because there is suggestive evidence that PBM therapy enhances blood flow, the third study aim will examine whether 12-weeks of PBM therapy improves cerebral blood flow (CBF) in older adults with dementia. The final study aim is to investigate the effects of PBM treatment on quality of life in older adults with dementia and on caregiver burden.

Ten pairs of older adults with dementia and their caregivers (CG) will be enrolled in a randomized, wait-list control trial. Five primary participants, PP, (i.e., individuals with dementia) will be randomized to start PBM treatment immediately with the Vielight Neuro device after the baseline psychometric and

MRI assessments. Five PP will be randomized to a "Delayed PMB" group that will not start treatment with the Vielight Neuro device until after the 12-week psychometric and MRI assessment. Cognitive function and quality of life will be assessed in all PPs at baseline, weeks 6, 12, 18, and 24. Caregiver burden, positive aspects of caregiving, depressive symptomology, and dementia-related behaviors in the PP will be assessed in all CGs at baseline, weeks 6, 12, 18, and 24. Neuroimaging measures will only be assessed in PPs at baseline and week 12.

VII. Study Flow:



VIII. Study Aims:
1) Replicate the findings of Saltmarche et al. (2017) that 12 weeks of PBM therapy improves cognitive function in patients with dementia.

2) Examine the effect of 12 weeks of PBM therapy on DMN functional connectivity in individuals with dementia.

3) Examine the effect of 12 weeks of PBM therapy on cerebral blood flow in individuals with dementia.

4) Examine the effects of 12 weeks of PBM therapy on quality of life in individuals with dementia and caregiver burden.

5) Determine if changes in cognition and quality of life are associated with changes in DMN functional connectivity and cerebral perfusion after 12 weeks of PBM therapy.

IX. Study Hypotheses:
1) Primary participants (PP) randomized to in the Immediate PBM group will show improved cognitive function after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared to PP randomized to the Delayed PBM group.

2) Primary participants (PP) randomized to in the Immediate PBM group will show increased functional connectivity within nodes of the DMN after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared to PP randomized to the Delayed PBM group.

3) PP randomized to Immediate PBM will show increased cerebral perfusion after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared PP randomized to the Delayed PBM group.

4) Improvements in cognitive function (Alzheimer's Disease Assessment Scale-cognitive subscale, ADAS-cog, and the Montreal Cognitive Assessment, MOCA) and Quality of Life in Alzheimer's Disease (QOL-AD) scores will be correlated with increases in DMN functional connectivity and increases in cerebral perfusion after 12 weeks of PBM therapy with the Vielight Neuro in PP randomized to Immediate PBM.

5) Caregivers in the Immediate PBM group will experience greater reductions in caregiver burden (Caregiver Burden Inventory, CBI) and depressive symptomology and improvements in positive feelings about caregiving (Positive Aspects of Caregiving) after 12 weeks compared to caregivers in the Delayed PBM group.

X. Study Population and Sample Size:
We will study 10 dyads/pairs of individuals with dementia and their caregivers for this pilot, proof-of-concept study. Five individuals with dementia will be randomized to start PBM treatment with the Vielight Neuro device immediately after baseline assessments (Immediate PBM). Five individuals with dementia will be randomized to the Delayed PBM group, which will not start PBM treatment with the Vielight Neuro device until after completing Week 12 assessments. The sample size was chosen because this is what can be supported with the REAC and Department of Radiology Seed Grant funds. Also, because the Saltmarche et al. (2017) pilot study suggested that cognitive gains achieved by the dementia patients cannot be maintained once PBM treatments are discontinued, we plan to offer the Vielight Neuro devices to primary study participants upon completion of all study procedures so that

they may continue to use Thus the sample size is also limited by the cost of the Vielight Neuro device ($875 for research).

XI. Recruitment and Screening:
Two main methods will be used to recruit potential participants for the study. First, study co-investigator Dr. Deborah Barnes will to help identify and refer of individuals with dementia and caregivers who have completed her Prevention of Loss of Independence through Exercise (PLIÉ) study, who meet study eligibility criteria**,** and who have consented to be contacted about future studies. Second, we will post flyers about the study at adult day programs in the San Francisco Bay Area and in public areas such as SF VAMC clinic waiting rooms and neighborhoods surrounding the study sites.

Legally Authorized Representatives (LARs) referred by Dr. Barnes will be contacted by research staff, who will describe the study in more detail and will obtain verbal consent to screen the LAR and/or caregiver. Those who are interested and eligible will be scheduled for an in-person appointment to obtain written consent. For potential study participants not referred by Dr. Barnes (e.g., they find out about the study through flyers posted in the community), initial eligibility will be determined by self-referral.

Potential eligibility for study participation will be determined by Dr. Chao, Dr. Barnes, and the research team members. Potential eligibility will be determined in the protocol under the inclusion/exclusion criteria sections. LARs referred by Dr. Barnes will be contacted by research staff who will describe the study in more detail and will obtain verbal consent to screen the LAR and/or caregiver. Those who are interested and eligible will be scheduled for an in-person appointment to obtain written consent. For potential study participants not referred by Dr. Barnes (e.g., they find out about the study through flyers posted in the community), initial eligibility will be determined by self-referral.

The initial screening will be performed by telephone and will include assessment of demographics and eligibility criteria. Specifically, the LARs of potential study participants who have expressed interest in being contacted about the study will be screened for eligibility over the telephone. Basic demographic and medical history information also will be collected after obtaining verbal consent. This information will be collected during screening in order to compare characteristics of those who choose to participate or not to assess for potential selection biases. Those who are eligible and interested will then be scheduled for an in-person consent visit. LARs may also serve as the study caregiver (CG) or LARs may designate someone else (e.g., other family member, paid caregiver) to play this role. If an alternate study CG is designated, they will be screened and consented separately or consented with the LAR and potential participant if possible.

XII. Consent Visit (in person, PP and LAR together): Informed consent will be obtained with the primary study participant (PP) and LAR together at a location of their choosing (e.g., their home or the SF VAMC). Detailed consent procedures are provided in the Informed Consent section. Because the caregiver (CG) will also undergo psychometric assessment, s/he will be asked to sign a separate consent form. If an alternate CG is designated by the LAR, s/he may be consented with the primary participant or at another time and place. After consent has been obtained, global cognitive function, quality of life, and a MRI of the brain will be acquired in primary study participants.

A research staff member will ask the potential PP a series of yes/no questions to assess whether consent information was understood. The wording of the questions will be varied to ensure that primary study participants cannot simply answer yes or no to all questions. Information not understood will be

discussed again and understanding will be reassessed. If the PP is unable to answer questions after two discussions, s/he will be considered unable to provide consent and will be asked to assent to study procedures by agreeing to have their LAR provide consent on his/her behalf. PP who appear to dissent to study procedures (e.g., shake heads no, appear agitated or distressed) will be considered ineligible for the study even if their LAR provides consent.

Because the purpose of this study is to investigate whether PBM treatment delivered via the commercially available Vielight Neuro device can cognitive function in individuals with dementia, it is likely that some participants (i.e., individuals with dementia) will no longer have the capacity to consent for themselves due to the nature of their condition. A surrogate may be necessary to: a) provide consent should it be determined that the study participant does not have the capacity to make decisions; b) provide reliable information regarding the participant's current level of functioning including mental and physical functioning, relevant to the study and c) to provide information on caregiver burden resulting from the loss of independence of the participant.
Participants will never be forced to participate in the study or any of the study procedures. In addition, assessment of outcomes will be discontinued if there is evidence of distress or lack of assent.

We will use the following hierarchy if the PP, LAR, or CG differs regarding their desire to continue participation:

1) If the PP wishes to stop participating in the study (i.e., no longer consents/assents), the dyad of PP/CG will be withdrawn, regardless of the preferences of the CG or LAR.
2) If the PP does not demonstrate capacity to consent and the LAR wants the primary participant to stop participating in the study (i.e., withdraws consent on their behalf), the dyad will be withdrawn, regardless of the preferences of the PP or the CG.
3) If the CG wishes to stop participating in the study but the PP wishes to continue and, if the PP does not demonstrate capacity to consent, the LAR agrees (e.g., paid CG no longer wishes to be involved), then the PP will be allowed to continue without the CG's involvement.

XIII. Inclusion/Exclusion Criteria:

Inclusion criteria for primary participants (PP): age $\geq$ 50 years, a diagnosis of dementia (preferably AD) by their neurologist, English language fluency, caregiver consent, and no contraindications for MRI.

Inclusion criteria for caregivers (CG): current provision of care to PP and ability to answer questions about the PP's behaviors, quality of life, and their own level of stress.

Exclusion criteria for PP: lack of assent to study procedures, contraindications for MRI, terminal illness (i.e., life expectancy < 1 year), started dementia medication (i.e., cholinesterase inhibitor or memantine) in the past 3 months or planning to start new dementia medication, current participation in another research study that could potentially confound current study (e.g., medication or behavioral intervention).

Exclusion criteria for CG: major neurological or psychiatric condition, terminal illness, evidence of cognitive impairment or inability to consent to study procedures.

XIV. Study Procedures:

Baseline Psychometric Assessment (with PP and CG separately). The baseline assessment will take place after the consent visit and prior to randomization. PPs and CGs will be assessed separately so that their answers do not influence each other. PPs will be directly assessed by research staff while CGs will be

provided with self-administered forms when feasible or may complete the assessment in person, by phone or mail-in.

Baseline Assessments in PPs:

- Cognitive function (Montreal Cognitive Assessment MOCA). Cognitive function will be measured using the Montreal Cognitive Assessment, MOCA). The MOCA's score range is the same as the MMSE (0–30), but has additional, more complex tasks including executive function. Items address orientation, drawing figures, processing speed, naming objects, memory, recall, attention, vigilance, repetition, verbal fluency, and abstraction.
- Alzheimer's Disease Assessment Scale -- Cognitive Subscale (ADAS-cog). Cognitive function will also be assessment with the ADAS-cog, one of the most commonly used outcome measures in dementia drug treatment trials and one of the measures considered by the Food and Drug Administration (FDA) for approval of dementia medications. It includes direct assessment of learning (10-word list), naming (objects), following commands, constructional praxis (figure copying), ideational praxis (mailing a letter), orientation (person, time, place), recognition memory and remembering test instructions. The ADAS-cog is scored on an 80-point scale, with higher scores reflecting worse cognitive function.
- Quality of life (Quality of Life Scale in Alzheimer's Disease, QOL-AD). The Quality of Life Scale in Alzheimer's Disease (QOL-AD) is a standard quality of life measure that asks parallel questions of affected individuals and caregivers and was used in our pilot study. Current quality of life is rated as poor (1 point), fair (2 points), good (3 points) or excellent (4 points) in 13 areas: physical health, energy, mood, living situation, memory, family, marriage, friends, self as a whole, ability to do chores around the house, ability to do things for fun, money, and life as a whole. Scores may range from 13 to 52 with higher scores reflecting better quality of life. Prior studies have found that the QOL-AD is a valid and reliable measure, with Cronbach's alpha of 0.84 for patient reports and 0.86 for caregiver reports and interrater reliability based on Cohen's kappa values >0.70.

Baseline Assessments in CGs:

- Caregiver burden (Caregiver Burden Inventory, CBI). The Caregiver Burden Inventory (CBI) is a standard measure that includes 24 items and 5 domains. Caregivers are asked to rate how often each statement describes their feelings (never, rarely, sometimes, quite frequently, nearly always). The total score may range from 0 to 96 with higher scores reflecting greater feelings of burden. The CBI has good internal consistency (Cronbach's alpha, 0.73-0.86), and it was sensitive to change in our pilot study.
- Participant quality of life (QOL-AD). As described above, the QOL-AD is a standard quality of life measure that asks parallel questions of affected individuals and caregivers and was used in our pilot study.
- Neuropsychiatric Inventory (NPI). Caregivers will also complete the NPI, which assesses the frequency, severity and level of distress caused by 12 common dementia-related behaviors (delusions, hallucinations, agitation/aggression, depression/dysphoria, anxiety, elation/euphoria, apathy/indifference, disinhibition, irritability/lability, aberrant motor behavior, sleep and appetite/eating). The NPI has good test-retest reliability (0.79-0.86) and good internal consistency (Cronbach's alpha, 0.87-0.88).
- Positive Aspects of Caregiving Scale is a standard measure that asks caregivers to rate their agreement/disagreement with 11 statements about positive aspects of caregiving on a 5-point Likert scale (disagree a lot ... agree a lot).

- Geriatric Depression Scale (GDS) short form assesses depressive symptoms in caregivers based on self-report. The GDS short form is a 15-item yes/no scale that is valid in people with and without dementia

Follow-Up Psychometric Assessments (PPs and CGs separately). Follow-up assessments will be performed at weeks 6,12, 18, and 24 for all PPs and CGs. The procedures and measures will be identical to those at baseline.

Baseline and follow-up neuroimaging measures: Neuroimaging outcome measures will be acquired from PPs at baseline and week 12 on a research dedicated 3T Siemens Skyra at the San Francisco VA Medical Center. The following scans will be acquired at baseline and the post-treatment on a research-dedicated Siemens 3 Tesla Skyra MRI scanner at the SF VAMC: (1) T1-weighted 3D whole brain gradient echo MRI: TR/TE/TI = 2400/2.14/1000 ms, 1x1x1 mm^3 resolution, with fat suppression; (2) bandwidth matched variable flip angle spin echo T2-weighted 3D whole brain SPACE (Sampling Perfection with Application optimized Contrasts using different flip angle Evolution) MRI TR/TE 3200/565 ms, 1x1x1 mm^3 resolution, without fat suppression. (3) T2-weighted fluid attenuated inversion recovery (FLAIR) for optimal segmentation of white matter lesions: TR/TE/TI = 6000/355/2200 ms, 1x1x1 mm^3 resolution, We will use prospective motion correction by Kineticor on the T1, T2, and FLAIR images. (3) Whole-brain task-free resting-state fMRI: T2*-weighted gradient echo-echo planar sequence with 2.5 x 2.5 x 3 mm^3 resolution and TR/TE = 3000/30 ms. Subjects will be instructed to keep their eyes open. (4) Arterial-spin labeled (ASL) perfusion MRI will be acquired with a 2D pulsed ASL (pASL) sequence using the Siemens product PICORE PASL sequence with the Q2TIPs technique for defining the spin bolus: TR/TE = 3400/12 ms, TI1/TI = 700/1900 ms, field of view (FOV) = 256 mm, 24 sequential 4-mm-thick slices with a 25% gap between the adjacent slices, partial Fourier factor = 6/8, bandwidth = 2368 Hz/px, and imaging matrix = 64 × 64. The first volume of the 105 ASL acquisitions will be used as the M0 image; the remaining 104 volumes will be used as 52 control-label pairs. Subjects will be instructed to keep their eyes open during the ASL scans. The total scan time is approximately 45 minutes, the same scan duration as the Alzheimer's Disease Neuroimaging Initiative (ADNI) imaging protocol on the Siemens MRI scanner. In our experience, even older individuals with dementia can remain in the MRI scanner with minimal movement for this amount of time. Moreover, we have developed a protocol where we invite the participants' caregivers to accompany them into the MRI scan room to alleviate any potential anxiety, which further reduces motion-related artifacts.

XV. Randomization: Dyads of PP and CGs be randomized into one of two groups. The Immediate PBM Group will begin treatment with the Vielight Neuro device upon completion of baseline assessments. Treatment will continue for at least 12 weeks. *Because results from Saltmarche et al. pilot study suggest that cognitive gains achieved by patients with dementia may not be maintained without continued PBM treatment,[9] PPs/CGs randomized to the Immediate PBM group will have the option of continuing PBM treatment after completion of Week 12 assessments.* The Delayed PBM Group will not begin treatment with the Vielight Neuro device until after completion of week 12 assessments.

XVI. Caregiver Device Training -- Prior to starting the PBM treatments, the CG will be trained how to position the Vielight Neuro device on the PP, how to control, how to clean, and how to maintain the Vielight Neuro device in a single 30 minute session. This training session will either take place at the SF VAMC or at the location of the PP/CG's choice (e.g., their home).

XVII. Treatment Diary/Log: To encourage adherence to the home-treatment PBM regimen, CGs will be instructed to log each treatment in the "Daily Home Treatment Journal." CGs will also be instructed to

note changes in the participants' memory, other cognitive processes, and general health conditions in the journal, which will be reviewed at bi-monthly "check-in" calls with the research staff.

XVIII. Confirmation of Correct Device Use -- After the first week of treatment, a research staff member will confirm that the CG knows how to properly use and maintain the Vielight Neuro device. During the "confirmation" appointment, the CG will be asked to demonstrate his/her understanding and ability to correctly place the device on the PP. Any errors will be noted by the study staff and the caregiver will be re-trained or corrected as necessary. The research study staff will also review the treatment diary with the CG to check treatment compliance and its recording. The CG and PP will be encouraged to ask any questions or provide comments on device use which will be noted by study staff.

XIX. Monitoring. To encourage adherence to the home-treatment PBM regimen, study research staff will call CGs every two weeks to "check-in" on how the treatments are going. The Daily Home Treatment Journals will be reviewed during these calls. In addition to monitoring adherence, clinical safety, and any adverse events, factors that might affect study outcomes including changes in medications, caregivers, or medical conditions will also be assessed during the "check-in" calls. CGs will be also asked about any changes to the PP's health status since the last contact "check-in" call, including any emergency room visits, hospitalizations, or injuries. Events that are serious and unexpected will be reported to the UCSF IRB within 5 working days. Co-interventions also will be assessed during the "check in" calls using a standardized questionnaire that asks about any changes since last contact that might affect study outcomes including changes in medications, caregivers, medical conditions, usual activities or other factors.

XX. **Risks and Benefits:** There are some potential risks associated with the study. Potential risks associated with undergoing a MRI include: 1) metal flying into the MRI scanner, 2) minimal discomfort associated with lying supine for MRI scans, 3) possible claustrophobia when lying in the scanner, and 4) possible annoyance due to excessive noise produced by the MRI. We will minimize these risk by doing the following: 1) risk of metal flying into the MRI will be minimized by careful screening of the subject and by the use of a metal detector that is just outside the door leading into the MRI room, 2) discomfort from laying supine in the MRI scanner will be minimized with padding, pillows, and blankets to make the subject as comfortable as possible, 3) subjects will have voice contact with the MR and technician throughout the MRI scan and may be removed from the MR scanner at any time upon their request, 4) noise will be reduced by earplugs and noise reducing head phones. Risks to confidentiality will be minimized as described above.

Risks associated with the baseline or follow-up assessments include psychological or emotional distressing. This risk will minimized by having research staff who administer baseline and follow-up assessments be carefully trained using standard procedures on strategies for working with individuals with dementia and their CG in a sensitive and respectful manner. PP and CG will not be forced to answer any questions. Staff will be trained to recognize and appropriately address signs of discomfort or stress when indicated (e.g., by taking breaks, rescheduling appointment, skipping sections that cause undue discomfort). PP will have the option to choose between private viewing (available only to research staff, auditors, and monitoring committee) or private/public viewing (available to private viewers and also public, such as being included in the training video, study website, and/or presentations).

The research is justified because the risks to subjects are very low and the knowledge that could be gained from the study may aid with development and implementation of therapies that enhance brain function and quality of life in individuals with dementia.

XXI. Compensation Method: PP and CG will not be paid for study participation. However, because the Saltmarche et al. pilot study suggests that cognitive gains achieved by individuals with dementia are not maintained without continued PBM treatment, we feel it would be unethical to take back the PBM devices after the study. Therefore, PPs will be offered the opportunity to keep and to continue using their Vielight Neuro devices once they complete all study procedures.

XXII. Institutional Review Board (IRB) Approval
Subject enrollment and study related procedures will NOT be initiated until written study approval has been obtained from the UCSF/SF VAMC IRB.

We do not anticipate adverse events to be device attributable with use of the Vielight Neuro device. The device predecessor is currently sold as a general wellness device worldwide with no major adverse events having been reported to date. It satisfies the criteria of an unregulated low risk device defined by the FDA policy, "General Wellness: Policy for Low Risk Devices." However, should any Adverse Events or Serious Adverse Events (SAEs) occur during the study, these will be reported to the UCSF IRB immediately, within 5 working days of the PI's awareness of the event using an iRIS Adverse Event Reporting Form.

All SAEs need to be followed until the event is resolved (with or without sequelae). The PI will decide if more follow up information is needed in case the event is not resolved at study completion.

XXIII. Data Protection:
Loss of privacy may occur if research data are viewed by individuals outside the research team. We will minimize the risk of loss of privacy by training all research staff to maintain data in a secure manner and not to discuss study participants outside the research team. Research data will be stored in locked file cabinets or on VA-approved devices (e.g., VA-encrypted laptops) or secure VA servers. Study forms will include unique ID numbers but not names or other identifying information whenever possible. No names or other information that could identify subjects will be used for research of the data. A key that links each subject to a code number will be maintained in a database that is password protected and inside a secure computer network. Only the PI and designated staff working on this project will have access to the key. Signed consent forms will be kept in a locked cabinet and electronic information will be password protected

Consent forms and HIPPA records may be shared with the UCSF IRB and VA regulatory agencies during regular audits. Information about a subject's MRI may be shared with his/her physician if MRI abnormalities found.

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STATISTICAL ANALYSIS PLAN

We will use a two-sample t-test on the baseline vs. week-12 differences in PP cognitive measures to examine between-group differences to test hypothesis 1: Primary participants (PP) randomized to in the Immediate PBM group will show improvements in cognitive function after 12 weeks of near-infrared
PBM therapy with the Vielight Neuro device compared to PP randomized to the Delayed PBM group.

We will also use a repeated-measures analysis of variance (ANOVA) to analyze the between-group differences in the ADAS-cog and NPI, with time (baseline, 6-, and 12-weeks) as the within-subject factor.

A functional connectivity analysis produced coefficients from a priori defined seed-voxel correlation with Pearson correlation analysis will be used. Fisher's r-to-z transformation will be used to convert correlation maps into z maps. Random effect models will be applied for second level analysis. A two-sample t-test on the baseline vs. week-12 differences between the Immediate PBM and Delayed PBM groups (Immediate [baseline-week 12] vs. Delayed [baseline-week 12]) will be used to examine the difference between the two groups for hypothesis 2: PP randomized to the Immediate PBM group will show increased functional connectivity within nodes of the DMN after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared to PP randomized to the Delayed PBM group.

We will also use the CONN-fMRI Functional Connectivity toolbox version 17 to analyze the functional connectivity data. Default pre-processing parameters will be used to address the possible confounding effect of head motion artifacts and white matter (WM) and cerebral spinal fluid (CSF) blood oxygen level dependent (BOLD) signal. Blood oxygen level dependent (BOLD) signal noise from the WM and CSF will be characterized with the principal component-based noise-correctio 'CompCor' method utilized in the CONN toolbox. Band-pass filtering will be performed with a frequency window of 0.008 to 0.09 Hz. Region of interest (ROI) to ROI functional connectivity maps will be created for each participant. The mean BOLD time series will be computed across all voxels within each ROI. Bivariate-regression analyses will be used to determine the linear association of the BOLD time series between each pair of sources. Each scan will be Hanning weighted. We will use a predefined spherical ROI (with a radius of 10mm) from the CONN-fMRI Functional Connectivity toolbox as the seed to create connectivity maps of the DMN in the posterior cingulate cortex/precuneus (PCC) region, MNI coordinates 1, -61, 38, based on prior studies. For ROI-to-ROI analyses, a peak voxel threshold of $p \leq 0.001$ and a cluster extent threshold of $p \leq 0.05$ will be set for bidirectional explorations of connectivity (i.e., positive and negative associations).

We will extract the average cerebral blood flow (CBF) values for each subject by averaging the CBF values within a priori ROIs. Next, we will use a two-sample t-test on the baseline vs. week-12 differences between the Immediate PBM and Delayed PBM groups (Immediate [baseline-week 12] vs. Delayed [baseline-week 12]) to examine between-group differences for hypothesis 3: PP randomized to

Immediate PBM will show increased cerebral perfusion after 12 weeks of near-infrared PBM therapy with the Vielight Neuro device compared PP randomized to the Delayed PBM group.

We will also use a repeated-measures multivariate analysis of variance (MANOVA) to analyze the between-group differences in arterial spin labeled (ASL) perfusion MRI perfusion data, with time and ROI (superior frontal, superior parietal, supramarginal) as the within-subject factors. The ROIs (superior frontal, superior parietal, supramarginal gyrus) correspond to the locations of the transcranial LED clusters of the Vielight Neuro PBM device.