Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Glymphatic Therapies

Evidence Summary

Enhancement of the glymphatic system may attenuate cognitive decline by clearing waste from the brain. Clinical evidence is limited, but early studies for surgical and non-invasive approaches show promise.

Neuroprotective Benefit:

Lymphatic regeneration/enhancement: Modulation of cervical or meningeal lymphatics via gene therapy, pharmacological, or surgical interventions is beneficial in preclinical studies, but there is very limited clinical evidence and the safety risks may be high.

Pharmacological augmentation of glymphatic function: The aquaporin 4 channel plays a key role in the function of the glymphatic system, but its chronic activation comes with systemic risks. Other drugs with glymphatic boosting potential may not be suitable for chronic use.

Stimulation approaches to augment glymphatic function*:* Non-invasive stimulation altering neuronal activity and arterial vasomotion enhances glymphatic flow in preclinical models, and pilot studies with imaging suggest the effects are translatable to humans. Durability of effects are unclear.

Deep sleep enhancement stimulation: Pilot studies suggest non-invasive stimulation can augment slow wave sleep, but whether these approaches have sustained impacts on cognition and brain waste clearance mechanisms still need to be determined.

Aging and related health concerns: N/A
The glymphatic system is based in the CNS. Modulation of peripheral lymphatics can benefit in the context of lymphedema.

Safety:
Lymphatic regeneration/enhancement: These approaches involve invasive procedures including CNS-directed drug delivery and surgery to the head and neck region, which includes risks for infection, cancer, and abnormal growth or leakiness of lymphatic or blood vessels.

Drug-mediated augmentation of glymphatic function: Aquaporin 4 is important for systemic water balance and must be dynamically regulated. Chronic activation could disrupt kidney function, or potentiate tumor growth and chronic pain. Dexmedetomidine is a sedative and not suitable for chronic use.

Electric/Sensory stimulation: Stimulation approaches are generally safe and well-tolerated in clinical studies. Side effects are usually limited to mild stimulation site irritation and headache, though long-term safety data is limited.

Availability: Clinical trials or preclinical research	**Dose**: Not established. Protocols to meaningfully enhance glymphatic activity have not been clinically validated or approved, to date.
Half-life: Varies	**BBB**: Varies. Some approaches need to act specifically within the CNS, while others act on peripheral targets, and some involve non-invasive stimulation.
Clinical trials: Some interventions, such as stimulation approaches have been clinically tested, typically in small pilot trials in the context of neurodegeneration or sleep disorders, though clinical evidence for impacts to glymphatic function is limited.	**Observational studies**: Declines in glymphatic function have been observed in the context of aging and neurodegenerative disease.

What is it?

The glymphatic system facilitates fluid exchange within the CNS, which plays roles in the clearance of extracellular waste products, movement of signaling molecules, and immune surveillance [1]. This is driven by the exchange of cerebrospinal fluid (CSF) with interstitial fluid, which is the fluid between cells in the tissue, within the perivascular space, also called the Virchow-Robin space. The CSF is driven into the perivascular space via arterial pulsatility, respiration, and CSF pressure gradients influenced by the activity of astrocytic water channels called aquaporin 4 [2]. The exchange between the CSF and interstitial fluid facilitates the transfer of waste products and antigens out of the interstitial fluid into the CSF which will eventually be drained into the cervical lymph nodes. The glymphatic system is most active during sleep, particularly during deep slow wave sleep. Dysfunction of this system can lead to the accumulation of fluid and waste products within brain tissue and result in altered immune responses. This system appears to be less efficient in the context of aging, which is thought to play a role in age-related neurodegenerative diseases [2].
Our understanding of this system largely arises from work in rodents, and the degree to which the system in rodents corresponds to humans remains controversial. This largely stems from the difficulty of assessing the glymphatic system in humans. A variety of imaging techniques have been developed to assess surrogate measures of glymphatic activity, including non-invasive techniques [3]. These will be critical for the evaluation of novel therapeutic approaches being developed to augment the activity of the glymphatic system to prevent and treat neurodegenerative disease. To date, most of these therapeutic approaches have only been conducted in preclinical rodent models [1; 4]. Some approaches have been clinically tested, particularly those related to the entrainment of specific brain waves, but their impacts to glymphatic function in humans have not yet been well characterized.

Neuroprotective Benefit:

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

The activity of the glymphatic system has been shown to decrease in the context of aging, and is thought to contribute to cognitive decline [1]. There are currently no interventions that have been clinically validated to enhance glymphatic function. However, many of the proposed glymphatic interventions show preclinical evidence of enhancing glymphatic activity and cognition, and in some cases have preliminary clinical evidence of preserving cognitive function.

There is observational evidence to support that preservation of glymphatic system activity protects against age-related cognitive decline. A retrospective review including 633 participants found that, in the context of aging, individuals with a higher degree of glymphatic system function, based on an indirect MRI-based measure of the glymphatic system called the diffusion tensor imaging along the perivascular space (DTI-ALPS) index, had lower odds of cognitive decline [5].
Another study found that polymorphisms in the aquaporin4 (AQP4) water channel, which plays an important role in fluid flow through the glymphatic system, were associated with amyloid burden in the brain, as detected by Aβ PET imaging, as well as rate of progression on cognitive decline [6]. This suggests that the efficiency of glymphatic-mediated clearance of amyloid (and other pathological proteins) could influence neurodegenerative disease trajectories.

Human research to suggest benefits to patients with dementia:

Several of the techniques described for enhancing glymphatic function have been tested in patients with mild cognitive impairment (MCI) or dementia, including 40 Hz gamma entrainment, transcranial electric stimulation, photobiomodulation, transcranial magnetic stimulation, and auditory stimulation [1]. However, the studies were designed to assess impacts of cognition and/or sleep and did not assess glymphatic function, such that the degree, if any, to which that augmentation of glymphatic function contributed to neuroprotective outcomes is currently unclear.

Therapeutic interventions aimed at boosting glymphatic system function:

Lymphatic regeneration/enhancement:

Neuroprotective Benefit: Modulation of cervical or meningeal lymphatics via gene therapy, pharmacological, or surgical interventions is beneficial in preclinical studies, but there is very limited clinical evidence and the safety risks may be high.

Types of evidence:

- 1 review of 9 studies involving surgical cervical lymphatic bypass procedures
- Numerous laboratory studies

(Meningeal) Lymphatic regeneration

The lymphatic system is a part of the immune system which regulates fluid balance in the body through a network of vessels and nodes [7]. Nutrient filled plasma flows from capillaries into tissues, while the fluid filled with leftover metabolic waste products is taken back up by lymphatic vessels. The waste products are filtered out within the lymph nodes, then the fluid is returned into the bloodstream. If the lymphatic system is not working properly, fluid and waste products can build up in the tissues, resulting in tissue damage. The glymphatic system, the fluid exchange and waste clearance system specific to the CNS, is connected to the network of the broader lymphatic system through the meningeal lymphatic vessels to the cervical lymph nodes [8]. CSF is drained in the dura mater allowing for exchange with the meningeal lymphatic vessels before returning to the circulation.

Like other parts of the lymphatic system, the meningeal lymphatics also play a role in the regulation of immune responses [8]. A variety of immune cells take up residence in the meninges. The draining of CNS antigens into the cervical lymph nodes facilitates immune surveillance, allowing for timely responses to infections as well as the maintenance of tolerance to self-antigens. If this draining process gets disrupted, the immune response in the brain can get dysregulated, resulting in the unchecked spread of pathogens or tumor cells, and/or inflammatory autoimmune responses.

There is evidence to suggest that many meningeal lymphatic vessels atrophy with age, which negatively impacts CSF drainage, and may play a role in the development of neurodegenerative disease [8]. Therefore, one proposed therapeutic approach is to promote the development or regeneration of meningeal lymphatic vessels. The development of lymphatic vessels is controlled by the VEGF-C-VEGFR3 signaling axis, thus augmenting this pathway, typically through the genetic expression of VEGF-C (Vascular Endothelial Growth Factor-C), has been utilized by a variety of preclinical models to stimulate the development and restoration of lymphatic vessels, including meningeal lymphatics [8].

VEGF-C:

Preclinical evidence of glymphatic enhancement: Administration of adenoviral vector (AAV) conjugated VEGF-C into the CSF-filled subarachnoid space (i.e. intracisternal injection) has been shown to promote the growth and function of meningeal lymphatics in mice [9]. In aged mice, this restoration of meningeal lymphatic function has been associated with alleviation of cognitive deficits. One study found that meningeal lymphatic dysfunction was associated with altered inhibitory synaptic transmission and driven by excessive cortical expression of the inflammatory cytokine IL-6, in aged mice (20 to 24 months old) [9]. Restoration of meningeal lymphatics, via intracisternal injection of AAV1-VEGF-C, reduced levels of IL-6 and restored excitatory-inhibitory balance in the brains of the aged mice.

This approach appears best suited as a prevention strategy, and is not likely to benefit as a treatment for acute conditions, since it takes time for the gene therapy to be expressed and for lymphatic vessels to grow. This is supported by a study in mice assessing prophylactic vs therapeutic administration of AAV-VEGF-C via intracisternal injection, in the context of the tMCAO stroke model [10]. Pretreatment, starting four weeks prior to tMCAO, had a protective effect reducing infarct volume and neurological deficits, accompanied by an increase in meningeal lymphatic vessel growth. Meanwhile, AAV-VEGF-C administration following tMCAO had no therapeutic benefit, and in some cases may exacerbate inflammatory damage.

Potential Caveats: This is a relatively invasive approach. In order to minimize the potential for systemic effects, expression needs to be targeted within the CNS. Administration using AAVs raises the risk for an adverse immune response in the brain. In addition to supporting the growth of lymphatic vessels, VEGF-C may also affect blood vessels, thus its expression could result in blood vessel enlargement or leakiness [11; 12]. To date, this is a useful tool for research use, but is not the most practical approach for clinical translation.

Mechanical scaffold:
Research studies have not yet been published regarding the use of scaffolds to support the production/regeneration of CNS-related (i.e. cervical or meningeal) lymphatics for neurodegenerative disease, though this method has been used with peripheral lymphatics in the context of lymphedema. A patent has been filed for the use of implantable scaffolds for modulating lymphatic activity in the CNS (Google Patents WO2024124057A1). This strategy is aimed at enhancing fluid flow (CSF and interstitial fluid) in the CNS, to help clear waste products from the brain, including toxic misfolded proteins, which may be beneficial in the context of neurodegenerative disease. The scaffold is designed to induce directional lymphangiogenesis, leading to an increase in CSF turnover of 1 to 10%. The scaffolds may be used to connect degrading efferent lymphatic vessels to cervical lymph nodes to try to enhance draining. They may also be embedded with VEGF-C, to facilitate lymphatic vessel production/regeneration.

Potential Caveats: Further studies are needed to determine the utility of this type of approach.

Enhancement of cervical (and nasal) lymphatics
The cervical lymphatic vessels are located in the head and neck, which drain into the cervical lymph nodes in the neck. In certain anatomical regions, the meningeal lymphatics connect with the cervical lymphatics to facilitate draining into the lymph nodes, such that impaired flow of the cervical lymphatic

system may slow or disrupt fluid drainage and waste clearance mechanisms in the brain [13]. The cervical lymphatics can be classified as superficial or deep. Both types ultimately drain to the deep cervical lymph nodes. Due to their location deep within the neck, deep cervical lymph nodes can only be accessed via invasive surgery, and thus have been considered difficult to target. Meanwhile, enhancement of the superficial lymphatics, such as those in the submandibular region, has emerged as a potentially less/non-invasive therapeutic target [14].

The nasal lymphatics, which move fluid along the olfactory nerves through the cribriform plate [15]. They represent a major pathway of CSF movement and outflow in rodent models, but the contribution of this pathway in humans is still controversial.

The lymphatic vessels are lined with smooth muscle cells which undergo contractions that drive the flow of lymphatic fluid [16]. The activity of these smooth muscle cells appears to diminish with age, and several interventions have been proposed to augment their function.

Prostaglandins:

Prostaglandin F2α and its analogs have been shown to increase smooth muscle contractility and promote the vessel contractility of lymphatic vessels [17].

Preclinical evidence of glymphatic enhancement: Topical application of prostaglandin F2α (dinoprost) to the exposed superficial cervical lymphatic vessels in mice restored vessel pulsation in aged mice and diminished backflow, resulted in enhanced CSF drainage to the deep cervical lymph nodes [18]. While this is an invasive approach, another study in mice tested the impact of nasally administered prostaglandin F2α analogs on CSF outflow via the nasal lymphatics [19]. Treatment with latanoprost dose-dependently (0.1, 0.5, or 1mg/mL) enhanced the outflow of a CSF tracer into the nasal turbinate tissue via the nasal lymphatic system. However, the translatability of this approach is unclear since the contribution of nasal lymphatics to CSF outflow, and importance relative to other pathways has not been conclusively determined in humans.

Of note, latanoprost is typically administered as an ophthalmic solution to lower intraocular pressure by increasing fluid outflow from the eye through the stimulation of ocular lymphatic drainage [20]. This pathway is distinct from the ocular glymphatic pathway, which facilitates fluid exchange and waste clearance in the perivascular spaces of the optic nerve [21]. Impairment of both pathways may contribute to glaucoma, and augmentation (such as with latanoprost) can be a beneficial therapeutic approach for this condition. However, there is no clear evidence to date to indicate that the stimulation of the ocular lymphatic (or glymphatic) system would meaningfully enhance fluid flow and waste clearance in the brain.

Potential Caveats: The clinical translatability remains to be determined, including a non-invasive delivery mechanism of the prostaglandins in a manner which stimulates lymphatic flow while minimizing systemic side effects. The relative importance of superficial cervical lymphatics and nasal lymphatics to CSF drainage and CNS fluid dynamics in humans still needs to be clarified.

Mechanical stimulation:
Preclinical evidence of glymphatic enhancement: A force-regulated mechanical device that manipulates (compresses) superficial cervical lymphatic vessels, such as the submandibular lymphatics, through the intact skin was found to increase CSF outflow and drainage in aged mice [14]. The study authors confirmed that this drainage pathway was also functional in non-human primates (Macaca fascicularis).

Potential Caveats: The clinical translatability remains to be determined, including the degree of compression and duration of stimulation needed to impact superficial cervical lymphatic vessels in humans, which will impact the feasibility of the approach. The durability of the response also needs to be established.

Surgical cervical lymphatic bypass procedures:
The redirection of lymphatic flow from functional lymphatic vessels to nearby veins to facilitate pressure differential mediated fluid drainage, known as lymphatico-venous anastomosis, is used as a surgical technique in the context of peripheral lymphedema [22].
Clinical studies: A review identified 30 reported cases of the use of deep cervical lympho-venous bypass procedures in the context of Alzheimer's disease (AD) and Parkinson's disease [22]. Although symptomatic improvements were noted, the authors of the review caution that most of these studies were lacking in methodological detail and had a high risk of bias. This is a rapidly growing research area, with numerous clinical trials, primarily conducted in China, testing these procedures in the context of neurodegenerative disease.

Potential caveats: This is a highly invasive surgical procedure with a variety of safety risks including infection [22]. The ability of this technique to enhance glymphatic flow has not been well validated.

Pharmacological augmentation of glymphatic function
Neuroprotective Benefit: The aquaporin 4 channel plays a key role in the function of the glymphatic system, but its chronic activation comes with systemic risks. Other drugs with glymphatic boosting potential may not be suitable for chronic use.

Types of evidence:

- 1 review of glymphatic-associated imaging outcomes for dexmedetomidine
- Numerous laboratory studies

AQP4 Facilitator

Aquaporin 4 (AQP4) is a bidirectional water channel that is highly expressed on the endfeet of perivascular astrocytes [23]. The perivascular spaces, the compartments surrounding blood vessels in the brain, are where the glial and vascular endothelial membranes are in close proximity. These perivascular spaces serve as a low resistance pathway for CSF flow into the brain parenchyma, which may facilitate the exchange between CSF and interstitial fluid [23]. The glial membrane interacting with the perivascular space, the astrocytic endfeet, express AQP4. The permeability of this channel to water appears to be influenced by pH, with greater permeability at low pH due to a conformational change mediated by a pH-sensitive residue [24]. Studies in mice have found that the loss of AQP4 leads to altered fluid transport in the brain including higher levels of interstitial fluid. AQP4 appears to be important for interstitial fluid exchange, such that the loss of the AQP4 channels hinders the flow of interstitial fluid into the perivascular space, thereby increasing the resistance for CSF flow and exchange [23]. As a result, AQP4 has emerged as a potential therapeutic target to enhance the activity of the glymphatic system.

Preclinical evidence of glymphatic enhancement: The compound TGN-073 (N-(3-benzyloxypyridin-2-yl)-benzene-sulfonamide) was developed in the lab of Tsutomu Nakada at the University of Niigata, Japan [25]. It was found to act as an AQP4 facilitator in preclinical models. In mice, treatment with TGN-073 (200 mg/kg i.p.) increased the turnover of labeled water ($[^{17}O]H_2O$) in the brain based on MR water tracer imaging, by enhancing the turnover of interstitial fluid from the cortex into the pericapillary perivascular space [25]. The tracer flux was not different in brain regions lacking abundant AQP4, and the gross transport of the labeled water from the blood to the CSF was also not affected. Similarly, a study in rats found that TGN-073 treatment increased water diffusivity (i.e. water flux) in the brain using diffusion-weighted imaging (DWI), and allowed for greater uptake and penetration within the brain of the contrast agent Gd-DTPA using serial 3D T1-weighted MRI [26]. The degree of tracer distribution varied across brain regions in a manner consistent with variation in AQP4 expression and glymphatic transport capacity.

Aβ Clearance: There is evidence from mouse models of AD and obesity to suggest that AQP4 facilitation may enhance the clearance of Aβ from the brain. In the APP/PS1 AD mouse model, treatment with the

AQP4 facilitator TGN-073 (200 mg/kg i.p.) for 28 days starting at two months of age led to a reduction in levels of soluble and fibrillar Aβ in the brain, which was accompanied by improved memory performance [27]. Meanwhile, treatment with an AQP4 inhibitor (TGN-020) led to an increase in Aβ levels. Similarly, in a mouse model of high-fat diet-induced obesity, treatment with the AQP4 facilitator (TGN-073) enhanced Aβ clearance from the brain via the glymphatic system, while use of an AQP4 inhibitor exacerbated the accumulation of Aβ [28].

Potential caveats: AQP4 is expressed and important for water balance throughout the body. Therefore, systemic use of an AQP4 facilitator could have adverse effects on other organ systems such as the kidneys [4]. Water balance needs to be finely regulated, so this type of agent could negatively impact outcomes in certain contexts, such as acute phases of brain injury/stroke.

Dexmedetomidine

Dexmedetomidine is a selective alpha-2 adrenergic agonist used clinically for sedation (see Dexmedetomidine report) [29]. It inhibits the release of norepinephrine by binding alpha receptors in the brainstem.

Preclinical evidence of glymphatic enhancement: Preclinical animal studies provide evidence to suggest that dexmedetomidine may act as a glymphatic enhancer [30]. Studies in rodents have reported that treatment with dexmedetomidine increased CSF influx, mitigated neuroinflammation, facilitated the re-polarization of AQP4 to astrocytic endfeet, and enhanced the clearance of metabolic waste products in the brain [31; 32]. The enhancement of fluid flow may be driven by arterial vasomotor changes. These effects would be in line with a recent study (described in gamma entrainment section) indicating that stimulation of alpha-2 adrenergic receptors (A2AR) promotes the polarization of AQP4 and enhancement of arterial vasomotion [33].

Evidence of glymphatic enhancement in humans: While the impact of dexmedetomidine on glymphatic activity has not yet been formally assessed in clinical studies, several studies in humans have found that it increases the arterial pulsatility index in cerebral arteries [30]. Since vasomotor force is an important driver of glymphatic flow, an increase in cerebral pulsatility would be expected to enhance fluid flow and clearance in the brain, under physiological conditions.

Potential caveats: Dexmedetomidine is a sedative typically used acutely in a hospital setting. As it is currently used, it would not be suitable for chronic use, thus its ability to have long term impacts on glymphatic function seems unlikely.

Stimulation Approaches

Neuroprotective Benefit: Non-invasive stimulation altering neuronal activity and arterial vasomotion enhances glymphatic flow in preclinical models, and pilot studies with imaging suggest the effects are translatable to humans. Durability of effects are unclear.

Types of evidence:

- 2 small clinical studies of 40 Hz stimulation with indirect glymphatic measures
- 3 small clinical studies of rTMS with indirect glymphatic measures
- Numerous laboratory studies

Gamma Entrainment

Exposure to sensory (visual, auditory, vibration) stimulation at a frequency of 40 Hz promotes the entrainment of gamma rhythms in the brain [34]. Numerous preclinical studies and early clinical studies provide evidence to suggest this type of entrainment has beneficial effects on brain function. Enhanced clearance of Aβ was one of the initial benefits noted for this type of intervention, which is thought to stem from enhanced waste clearance from the brain via the glymphatic system [35].

Mechanism: In addition to promoting sleep [36], studies in mice indicate that 40 Hz stimulation can also enhance the activity of the glymphatic system in a sleep-independent manner [33]. 40 Hz stimulation using 3000 lux LEDs with cycles of 12.5 ms ON and 12.5 ms OFF for 30 minutes increased CSF tracer distribution in the brain, which is indicative of enhanced glymphatic flow [33]. The effect appears to be related to stimulation of the adrenergic system, particularly the activation of adenosine-A2A receptor (A2AR) signaling [33]. Adenosine is released from neurons in an activity-dependent manner. Adenosine released into the extracellular space via the transporter ENT2 following 40 Hz stimulation, triggers A2AR signaling, leading to the polarization of AQP4 along the astrocytic endfeet and enhancement of arterial vasomotion. Another group found that 40 Hz-induced glymphatic clearance was driven by enhanced arterial vasomotion and AQP4 polarization related to that activity of vasoactive intestinal peptide interneurons [37], which have been shown to be important for modulating extracellular adenosine levels. Together, these events facilitate fluid exchange within the perivascular spaces, thereby enhancing glymphatic-mediated clearance.

Evidence of glymphatic enhancement in humans: There have been a few pilot studies assessing the effect of 40 Hz stimulation on blood oxygenation level-dependent (BOLD) activity using fMRI, which is used to indirectly measure brain activity and blood flow. The relationship between the BOLD signal and

CSF signal, referred to as BOLD-CSF coupling, has been used as an indirect measure of glymphatic activity [38]. One study found that 40 Hz tACS stimulation to the dorsolateral prefrontal cortex, led to increased BOLD signal in that region, but it did not assess BOLD-CSF coupling [39]. Another study found that 40 Hz transcranial vibration stimulation augments spontaneous brain activity and global BOLD-CSF coupling, suggestive of a synchronization between brain activity and CSF dynamics [40]. Notably, stimulation at 30 Hz and 50 Hz did not impact BOLD-CSF coupling, indicating the effect is specific to 40 Hz neural modulation.

The 40 Hz stimulation device from Cognito Therapeutics is currently being tested in the randomized, double-blind, sham-controlled Phase 3 HOPE Study (NCT05637801) in patients with mild to moderate AD, with outcome measures related to cognition and brain atrophy. However, the study is not designed to assess the contribution of glymphatic modulation to these outcomes.

Vagal nerve stimulation

The vagus nerve is a cranial nerve that connects the brain to other parts of the body, playing an important role in communication between the brain and periphery. It also plays a critical role in the regulation of the autonomic nervous system. Vagal nerve stimulation (see Vagal Nerve Stimulation report) is a therapeutic technique involving neuromodulation by stimulating the vagus nerve with electrical signals. Implantable devices have been used for epilepsy, while more recent non-invasive devices are being tested in a range of conditions associated with autonomic dysfunction.

Preclinical evidence of glymphatic enhancement: Preclinical animal studies provide evidence that vagal nerve stimulation, including non-invasive methods, may enhance CSF flow. The effect is thought to stem from the vagus nerve's influence on cardiopulmonary activity, since arterial pulsatility and respiratory are key drivers of glymphatic flow. Stimulation cycles from surgically implanted vagal nerve stimulators increased the penetrance of CSF tracers through the brain in mice [41]. Non-invasive vagal stimulation, including transcutaneous auricular vagus nerve stimulation, was also found to enhance vasomotion and the flux of CSF tracers in rats and mice [42; 43].

Evidence of glymphatic enhancement in humans: To date, clinical trials using these devices have not yet assessed their impact on glymphatic function, though one such study is underway.

Repetitive transcranial magnetic stimulation (rTMS)

Transcranial magnetic stimulation is a non-invasive technique used to modulate neuronal activity in particular brain regions through the production of a magnetic field (see Transcranial Magnetic

Stimulation report) [44]. The rapid magnetic pulses produce an electric field that can influence neuronal excitability in the brain. It is used clinically in the treatment of various neuro-psychological disorders. rTMS involves the delivery of long trains of pulses lasting for several minutes.

Evidence of glymphatic enhancement in humans: There is evidence from preclinical animal studies, as well as preliminary data from clinical studies to suggest that one of the neuroprotective mechanisms of rTMS involves the augmentation of the glymphatic system. For example, in the 5XFAD mouse model, rTMS enhanced the clearance of CSF tracer from the mouse brain, indicative of increased glymphatic activity [45]. The clinical studies primarily assessed glymphatic system activity through the non-invasive MR imaging technique called diffusion tensor imaging analysis along the perivascular space (DTI-ALPS). One study tested the effect of 10 sessions of low-frequency rTMS in individuals with chronic insomnia over the course of two weeks [46]. Increases in the DTI-ALPS index, indicative of increased glymphatic flow, were apparent in rTMS treated participants by three months of follow-up. Moreover, the degree of improvement in DTI-ALPS (glymphatic activity) was correlated with the degree of improvement on metrics related to sleep quality and cognitive performance.
The impact to glymphatic function, based on the DTI-ALPS index, of a subtype of high-frequency rTMS called theta burst stimulation has also been tested. A small exploratory study assessing the effect of 10 sessions of continuous theta burst stimulation to the right dorsolateral prefrontal cortex over two weeks in patients with cerebral small vessel disease found that increases in glymphatic activity were more apparent after stimulation [47]. However, an increase in glymphatic activity, based on the DTI-ALPS index, was not observed in patients with MCI or mild AD in an RCT testing the effect of 10 sessions of intermittent theta-burst stimulation applied to the left dorsolateral prefrontal cortex over two weeks [48].
Potential caveats: It is not clear whether differences across studies stem from differences in study population, stimulation protocols, assessment window, imaging protocols, or a reflection of noise due to small sample sizes. To date, no stimulation protocols have been developed that have been optimized for the activation of the glymphatic system.

Deep Sleep Enhancement Stimulation

Neuroprotective Benefit: Pilot studies suggest non-invasive stimulation can augment slow wave sleep, but whether these approaches have sustained impacts on cognition and brain waste clearance mechanisms still need to be determined.

Types of evidence:

- 1 review of clinical trials testing photobiomodulation in neurological diseases
- 2 clinical studies assessing impact of photobiomodulation on indirect glymphatic measures
- 1 pilot clinical study assessing TES for deep sleep enhancement
- 1 pilot clinical study assessing auditory stimulation for deep sleep enhancement
- Numerous laboratory studies

Slow wave sleep, also called deep sleep (stage N3), is a period of sleep characterized by slow frequency (0.5-4 Hz) and high amplitude (> 75 µV) delta waves, as measured by electroencephalography (EEG) [49]. This period of sleep is important for memory consolidation and is the stage when waste clearance via the glymphatic system is most active [1]. The delta waves drive CSF flow into the perivascular spaces, facilitating fluid exchange, and clearance of cellular waste products from the interstitial fluid. Slow wave sleep declines in the context of aging, which negatively impacts waste clearance in the brain, and may negatively impact cognitive function. Therefore, one method to promote glymphatic activity is by enhancing slow wave sleep. One strategy to enhance slow wave sleep is through the entrainment/enhancement of the slow waves. Various different modalities have been tested, including light waves, sound waves, and electrical stimulation. Several studies have found that slow oscillations originate in the frontal cortex and then propagate to other brain regions [50], thus stimulation has most often been targeted to the frontal cortex. However different techniques and devices will have different degrees of precision, and the optimal location and stimulation parameters for the facilitation of the glymphatic system are not yet clear.

Transcranial photobiomodulation;
Photobiomodulation generally refers to the application of red or near-infrared light (600-1100 nm) to a certain part of the body [51]. Transcranial photobiomodulation involves the use of a helmet or headband with LED lights, typically aimed at targeting the frontal cortex in a non-invasive manner. Various neuroprotective benefits have been proposed, including the enhancement of deep sleep and glymphatic clearance. Various preclinical models have found that photobiomodulation promotes stimulation of the lymphatic and glymphatic systems, though the mechanisms have not been fully elucidated [52]. Some proposed mechanisms include enhancement of cerebral blood flow and augmentation of mitochondrial function [52].

Clinical studies effect on sleep: Several small clinical studies, primarily in conditions of neurodegenerative disease or brain injury, have found that transcranial photobiomodulation may

enhance deep sleep and positively impact cognitive function [52]. Due to the technical challenges of visualizing the glymphatic system and monitoring its activity in humans, these studies looking at the effects on sleep generally did not assess the impact to the glymphatic system.

Evidence of glymphatic enhancement in humans: There have been some preliminary studies assessing the impact of photobiomodulation on CSF flow via indirect measures, independent of its effects on deep sleep. One study assessed the effect of transcranial photobiomodulation on CSF flow in healthy young adults using blood-oxygenation level-dependent (BOLD) fMRI testing wavelengths of 808 nm and 1064 nm and pulsation frequencies of 10 Hz and 40 Hz [53]. The study found that the BOLD fMRI signal in CSF-related regions of interest following photobiomodulation are consistent with enhanced CSF drainage. The effect was influenced by skin melanin content. Another study used two-channel broad-band near-infrared spectroscopy (NIRS) to assess hemoglobin and free water dynamics in CSF in response to transcranial photobiomodulation (800 nm) targeted to the prefrontal cortex [54]. They found preliminary evidence of changes to CSF coupling in response to photobiomodulation, and that this relationship was altered in the context of aging and neurodegenerative disease (AD).

Potential caveats: Simulation parameters have not yet been optimized for the enhancement of glymphatic clearance. Due to the potential influence of skin melanin content, stimulation parameters, such as wavelength, intensity, and frequency may need to be personalized. The durability of the responses have not been established, such that the frequency of stimulation (and whether that is realistically feasible) needed to have a meaningful effect on the glymphatic system is not yet known.

Transcranial electrical stimulation (TES):
The non-invasive neuromodulation technique of TES involves the application of current through electrodes placed on the scalp with the goal of modulating brain activity [55]. The most common types are tACS and tDCS (see Transcranial Direct Current Stimulation report).
Clinical studies effect on sleep: One research group found that rather than the frontal cortex, slow oscillations appear to originate in the anterior ventral limbic cortex [56]. They performed a small study using TES applied to frontopolar and inferior frontal head sites with the goal of synchronizing limbic sources of slow oscillations and assessing the ability of this technique to enhance the duration of slow wave sleep [56]. The small study utilized a TES stimulation protocol of a 0.5 Hz sine wave with 520 µA total current administered in five blocks with a one-minute rest in between. Sleep stages were monitored using EEG, and the stimulation was initiated after participants showed evidence of stable N2

sleep. TES synchronized slow oscillations in N3 sleep and enhanced the duration of N3 following stimulation. Increases in the slow wave power band were most prominent in the medial frontal region.

Evidence of glymphatic enhancement in humans: The impact of this technique on the glymphatic system has not yet been assessed.
Potential caveats: Whether this technique can reliably enhance glymphatic system activity and brain waste clearance in humans is not yet clear. The stimulation parameters and frequency of intervention needed to have durable effects have also not been established.

Auditory stimulation:
Phase targeted acoustic stimuli have been shown to boost slow waves in a variety of research studies.
Clinical studies effect on sleep: One research group developed a portable sleep monitoring and feedback-controlled slow wave modulation device (MHSL-Sleepbandv2) for in-home use to assess the ability of auditory stimulation to enhance slow wave activity in healthy older adults (aged 60-80) without sleep disorders (NCT03420677) [57]. The stimulation (up-phase targeted phase-locked loop 1 Hz) was triggered based on EEG data characteristic of slow wave sleep, and volume was adapted to the depth of sleep to prevent arousal, ranging from 46 dB to 60 dB. The six-week, randomized, crossover study was terminated prematurely due to complications from the pandemic, but an interim analysis of 16 participants found that stimulation increased the spectral power in the low-frequency slow wave range between 0.75–1.25 Hz [57].

Potential caveats: Response was variable across participants, depending on the number of N3 bouts, as those with fewer slow wave bouts had fewer stimulations, and a weaker overall response [57]. This suggests that this method could potentially be less effective in individuals experiencing pronounced slow wave sleep deficiencies and sleep fragmentation, though more work is needed to determine if this could be resolved using a different stimulation paradigm.
Evidence of glymphatic enhancement in humans: It has not yet been established whether this method can reliably augment glymphatic system activity in humans.

APOE4 interactions: There is evidence to suggest that meningeal lymphatic function may be influenced by APOE4, such that meningeal lymphatic vessel shrinkage and leakage may be exacerbated with age in APOE4 carriers, resulting in reduced fluid flow [58]. This suggests that APOE4 carriers may preferentially benefit from glymphatic enhancers, and could potentially be less responsive to interventions that require intact meningeal lymphatic function.

Aging and related health concerns: ***Rated N/A for potential and N/A for evidence.***

Types of evidence:

- 2 clinical trials testing Lymfactin for lymphedema
- 3 review/case series using BioBridge for lymphedema
- Several laboratory studies

Since the glymphatic system is a mechanism for the clearance of metabolic and other waste products from the CNS via fluid exchange and transport to draining lymph nodes, its modulation is unlikely to be directly relevant for peripheral age-related diseases. Enhancing the glymphatic system may benefit longevity via the preservation of neurological function, though further experimental validation is needed. One of the therapeutic mechanisms proposed to enhance glymphatic function, namely, the promotion of lymphatic vessel growth, has been tested in the context of peripheral lymphedema, and may provide some evidence with respect to the prospective utility of these approaches for glymphatic modulation.

Lymphedema

The clinical indication in which stimulation of lymphatic vessel growth has been tested is breast cancer-related lymphedema as part of a treatment strategy that includes the surgical techniques lymph node transfer or venolymphatic anastomosis (lymphatic vessel re-routing). The two clinically tested strategies include induction of the genetic program for lymphangiogenesis via VEGF-C, and the use of a bio-compatible scaffold to support lymphatic regeneration.

Lymfactin® is a gene-therapy developed by Herantis Pharma designed to induce expression of human VEGF-C. This therapy is induced into cells using the adenovirus type 5 (AAV5) viral vector, which has broad tropism. It was tested at a dose of 1×10^{10} viral particles in three patients and at a dose of 1×10^{11} viral particles via perinodal injection in 12 patients with breast cancer-associated secondary lymphedema of the upper arm in combination with microvascular lymph node transfer surgery in a Phase 1 trial [59]. Participants in this open-label study reported improvements on the Lymphedema Quality of Life Inventory. There was a decrease in median excess volume with compression at 12 months, but no further improvement at 24 months follow-up [60]. However, the benefit of Lymfactin® in combination with surgery relative to surgery alone was not confirmed in a placebo-controlled Phase 2 trial in 39 patients with breast cancer-related lymphedema [61]. Excess arm volume was reduced by at

least 25% in approximately half of participants from both study groups by 12 months. However, baseline differences in arm volume and quality of life measures between the study groups impacted the ability to reliably assess treatment outcomes (Press release). One measure, the tissue dielectric constant ratios, which assesses skin interstitial fluid levels, suggests Lymfactin® may have had a modestly positive impact on the lymphatic system [61]. In preclinical studies, expression of VEGF-C for one to two weeks was sufficient to regenerate damaged lymphatic vessels [59], but this degree of regeneration has not translated to clinical studies, thus far. Altogether, this approach did not confer meaningful clinical benefit, and clinical development of Lymfactin® by Herantis has been discontinued.

BioBridge™ is an aligned nanofibrillar collagen scaffold designed to mimic the endogenous collagen matrix composed of biocompatible and biodegradable mesh [62]. It was developed by Fibralign Corporation. BioBridge™ is placed percutaneously in the subcutaneous plane, scaffolding tracks were created by tunneling the scaffold in tandem from the site of intact lymphatic vessels distally to the site of surgical nod transfer, and then to the nearest intact nodal basin [63]. Retrospective reviews and case reports provide evidence to support its utility in supporting lymphatic vessel growth and reducing excess fluid in the context of lymphedema. A retrospective review including 14 patients with stage II-III lymphedema treated with a combination of liposuction, surgical procedures, and BioBridge™ found that the addition of BioBridge™ provided further fluid reduction [64]. The total edema volume reduction was 95% (±28%) with liposuction plus surgery. The total edema volume reduction increased to 103% (±31%) following placement of the BioBridge™. Case reports of three patients with primary lymphedema treated with surgical procedures (lymph node transfer or venolymphatic anastomosis) and implantation of the BioBridge™ scaffold including non-contrast magnetic resonance lymphography show evidence of excess fluid reductions as well as the appearance of new lymphatic structures in the region of the lymph node surgery [62]. In comparison, new vessel generation was not apparent in patients who underwent this type of surgery without the implantation of the BioBridge™ scaffold. Preclinical rodent studies show evidence that this approach can facilitate the formation of new lymphatic vessels [63]. The magnitude and durability of the impact of BioBridge™ in relation to surgical interventions alone needs further clinical validation. It is currently being tested in a controlled trial in combination with lymph node transfer in patients with breast cancer-associated lymphedema (NCT04606030).

Lymphatic regeneration/enhancement: POTENTIALLY HIGH RISK

Safety: These approaches involve invasive procedures including CNS-directed drug delivery and surgery to the head and neck region, which includes risks for infection, cancer, and abnormal growth or leakiness of lymphatic or blood vessels.

Types of evidence:

- 1 review of 9 studies involving surgical cervical lymphatic bypass procedures
- 2 clinical trials testing Lymfactin for lymphedema
- 3 review/case series using BioBridge for lymphedema
- Numerous laboratory studies

This is the riskiest approach since it requires invasive techniques, such as viral vector-mediated gene expression within the brain, surgical implantation of mechanical scaffolds or surgical redirection of lymphatic connections in the head/neck region.
Animal studies testing ***AAV-VEGF-C*** therapy utilize intracisternal injection [9], which is a risky technique due to its close proximity to the brainstem. Drug delivery into the CSF in humans is typically administered via intrathecal injection, which also carries a lot of risks [65]. This type of therapy also raises risks related to use of the viral vector, including adverse immune reactions [66], as well as spill-over effects for VEGF-C which could result in excessive growth of blood vessels, vessel leakiness, or promote tumor growth and spread by supporting tumor angiogenesis or lymph node metastasis [12; 67].
Surgical cervical lymphaticovenular anastomosis procedures should be considered high risk, particularly since this technique has not yet been fully optimized for use in the CNS and is still considered experimental. Risks include venous blood backflow into the CNS, transmission of pathogens into the CNS, as well as metastasis of head and neck tumors [22].
The use of these techniques for peripheral lymphedema has not been associated with an increase in adverse events in small clinical trials/case studies [59; 60; 61; 64]. However, application of these techniques within the CNS inherently involves higher risk.

Pharmacological augmentation of glymphatic function: RISK FOR SYSTEMIC SIDE EFFECTS

Safety: Aquaporin 4 is important for systemic water balance and must be dynamically regulated. Chronic activation could disrupt kidney function, or potentiate tumor growth and chronic pain. Dexmedetomidine is a sedative and not suitable for chronic use.

Types of evidence:

- 1 review of clinical studies (executive summary) for dexmedetomidine
- Numerous laboratory studies

AQP4 facilitators have not yet been clinically tested, and the animal studies conducted thus far have not focused on safety outcomes [25]. However, long-term use of AQP4 facilitators could impact water balance in a variety of organ systems, which may negatively impact their function, particularly in the kidneys or eyes [4]. In certain contexts, inhibition of AQP4 may be more clinically appropriate, such as the case of acute cerebral edema, stroke, and brain injury. Dysregulation of AQP4 has been implicated in cancer, such that AQP4 inhibitors show anti-tumor effects in some model systems, suggesting that AQP4 facilitation may potentiate tumor growth [68]. AQP4 is also implicated in chronic pain, such that AQP4 could potentially exacerbate pain in some types of inflammatory and neuropathic pain [69].

Dexmedetomidine: As a sedative typically administered acutely in a hospital setting, this type of mediation would not be amenable to chronic use. In cardiac surgery patients, perioperative dexmedetomidine decreases mortality, but it may increase bradycardia and hypotension. Numerous drug interactions are known.

Electric/Sensory Stimulation: EXPECTED TO BE RELATIVELY SAFE

Safety: Stimulation approaches are generally safe and well-tolerated in clinical studies. Side effects are usually limited to mild stimulation site irritation and headache, though long-term safety data is limited.

Types of evidence:

- 3 clinical trials of 40 Hz stimulation in AD patients
- 2 clinical trials/reviews of dose-dependent safety of transcranial photobiomodulation
- 1 review of clinical studies (executive summary) for TMS
- 1 review of clinical studies (executive summary for tDCS)
- 1 review of clinical studies (executive summary) for Vagal nerve stimulation
- Numerous laboratory studies

Gamma entrainment: 40 Hz sensory stimulation has been safe and well-tolerated in small clinical studies to date. In patients with mild to moderate AD, adverse events were similar between treatment and sham groups in response to treatment with Cognito's Evoked Gamma Therapy System [70].

Similarly, other pilot trials testing 40 Hz sensory (visual and auditory) stimulation in healthy volunteers and patients with mild AD have found it to be safe and well-tolerated, with no significant adverse events [71].

Repetitive transcranial magnetic stimulation: rTMS is associated with mild and transient adverse events including headache, skin or scalp discomfort or tingling, neck pain, fatigue, and dizziness. Serious adverse events are very rare, though can include seizure, particularly in at-risk populations.

Transcranial photobiomodulation: Clinical studies testing transcranial photobiomodulation have shown strong safety in both healthy and patient populations. Furthermore, studies aimed at assessing the safety and tolerability of this method have found that treatment-emergent adverse events do not increase with repeated sessions [72; 73]. Vital signs, such as blood pressure, are not significantly affected [72]. Side effects are rarely reported, and those noted, including headache or dizziness, were considered mild [73].

Transcranial electric stimulation: tDCS may be accompanied by mild side effects at the stimulation site, though long-term effects are not known.

Vagal nerve stimulation: tVNS is generally considered safe for people without cardiac abnormalities, with stimulation site irritation as the most common side effect. Further studies aimed at optimizing stimulation parameters are needed.

Drug interactions: Interactions will vary depending on the approach and have not been fully characterized for most of these experimental interventions. Stimulation approaches may interact with drugs that affect neuronal excitability.

Sources and dosing:

To date, none of these methods have been approved or clinically validated to augment glymphatic function. Some of the stimulation devices, such as vagal nerve, photobiomodulation, TES, TMS, can be purchased from commercial retailers, however, the stimulation parameters related to the enhancement of glymphatic activity are still being clinically tested, and have not yet been optimized or standardized.

Research underway:

Most of the clinical studies with outcomes aimed at assessing glymphatic function are more focused on the validation of different imaging techniques and examining how the glymphatic activity is altered in different conditions. However, there are several studies examining the impact of therapeutic interventions on glymphatic activity, typically assessed via indirect imaging-based methods.

Non-invasive stimulation-based approaches

40 Hz gamma entrainment: One clinical trial assessing the effect of gamma entrainment using sensory stimuli (GENUS) for dementia prevention in adults 55+ at risk for Alzheimer's disease with evidence of brain amyloid will also look at the effect on glymphatic function (NCT05776641).
One clinical trial will assess the effect of 40 Hz light stimulation on glymphatic function in patients with Parkinson's disease (NCT06848621).

rTMS: Two clinical trials are assessing glymphatic function in response to TMS in patients with MCI (NCT07192913) or Alzheimer's disease (NCT06385106).

Photobiomodulation: One clinical trial will assess glymphatic function in response to near-infrared light therapy directed to the trigeminal nerve in healthy volunteers and patients with mild traumatic brain injury (NCT07044596).

Vagal nerve stimulation: One clinical trial will assess the ability of non-invasive vagal nerve stimulation (with gammaCore Sapphire) to stimulate amyloid clearance, via the glymphatic system, in patients with Cerebral Amyloid Angiopathy (NCT06421532).

Deep sleep enhancement/auditory stimulation: One clinical trial will assess the ability of multi-night closed-loop auditory stimulation administered during sleep to enhance slow wave sleep and glymphatic function in healthy young and older adults as well as older adults with SCD or MCI and evidence of brain amyloid (NCT07051239).

Surgical cervical lymphatic bypass procedures:
There are several studies, at least 7 active trials, examining the effects of surgical lymphatic bypass procedures, including lymphatic-venous anastomosis and deep cervical lymphovenous bypass, on

glymphatic function in several different neurodegenerative disease patient populations, including Alzheimer's disease (NCT06448442; NCT06852352; NCT06530732; NCT06448975; NCT06965062) multiple system atrophy (NCT07036939), and amyotrophic lateral sclerosis (NCT06351735). These studies are primarily being conducted in China.

Search terms:

Pubmed, Google: Glymphatic, VEGF-C, meningeal lymphatics, cervical lymphatics, deep sleep enhancement

- Alzheimer's disease, neurodegeneration, cognition, slow-wave sleep, safety, clinical trials

Websites visited for Glymphatic system enhancement:

- Clinicaltrials.gov (Gymphatic system)

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