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.

THN391

Evidence Summary

Higher fibrin(ogen) levels correlate with cognitive decline and dementia risk. The murine version of the antibody showed neuroprotective effects in mouse models of AD and MS. A phase I study is ongoing.

Neuroprotective Benefit: No studies have tested the neuroprotective benefit of THN391 in humans. Fibrin(ogen) levels are correlated with cognitive decline and dementia risk. The murine 5B8 antibody was neuroprotective in mouse models of AD and MS.

Aging and related health concerns: No studies have tested THN391 in age-related diseases. In a mouse model of COVID-19, the murine 5B8 antibody reduced macrophage activation and oxidative stress in the lungs.

Safety: A phase I study in healthy subjects is ongoing, as of October 2023. Based on interim findings, THN391 is well-tolerated with no serious adverse events. Detailed results have not been published in a peer-reviewed journal.

Availability: under clinical development	**Dose**: not established; administered intravenously	**Chemical formula:** N/A **MW**: not documented
Half-life: ~50 days	**BBB**: The murine version of the monoclonal antibody, 5B8, is penetrant in mice.	
Clinical trials: A phase I trial of single- and multiple-ascending dose studies is ongoing, as of Oct 2023.	**Observational studies**: None with THN391; numerous observational studies suggest high CSF and plasma fibrinogen levels are associated with increased risk for dementia.	

What is it?

THN391 is an anti-fibrin antibody under development by Therini Bio, Inc. for the treatment of dementia and other inflammatory neurodegenerative diseases such as multiple sclerosis. Fibrin is an insoluble protein that is a major component of a blood clot. Upon blood-brain barrier disruption, fibrinogen extravasates into the central nervous system tissue and is cleaved by thrombin into insoluble fibrin, a key proinflammatory matrix that activates innate immune responses (reviewed in Kantor et al., 2023). Fibrin is deposited in Alzheimer's disease brains as well as in multiple sclerosis lesions at sites of microglial activation and macrophage infiltration (Ryu et al., 2018).

THN391 is a humanized affinity-matured monoclonal antibody derived from murine monoclonal antibody clone 5B8 (Kantor et al., 2023). Conversion of fibrinogen to fibrin by thrombin exposes a cryptic epitope on the fibrinogen γ chain, 377–395, called P2, which can bind CD11b/CD18 receptor (also known as the complement receptor 3, or CR3) and CD11c/CD18 receptor (CR4) on microglia, macrophages, and dendritic cells, and trigger an inflammatory response (e.g., microglial and macrophage activation), leading to oxidative stress and secretion of cytokines that damage neurons. The P2 epitope is "cryptic" in soluble fibrinogen and is exposed only upon conversion of fibrinogen to insoluble fibrin. THN391 blocks binding of the CD11b and CD11c αI domain to the fibrin P2 epitope.

The P2 epitope involved in inflammation is spatially and compositionally distinct from the coagulation epitope (γ400-411; binding site for the platelet αIIbβ3 integrin receptor, required for platelet aggregation). The Fc region of THN391 was engineered to remove FcγR binding, making THN391 a purely antagonistic agent without the ability of mediating antibody effector functions (i.e., antibody-dependent cell killing/phagocytosis).

Neuroprotective Benefit: No studies have tested the neuroprotective benefit of THN391 in humans. Fibrin(ogen) levels are correlated with cognitive decline and dementia risk. The murine 5B8 antibody was neuroprotective in mouse models of AD and MS.

Types of evidence:

- 1 phase I trial of THN391
- Several observational studies examining the relationship between fibrinogen levels and dementia risk
- Several laboratory studies testing the murine monoclonal antibody 5B8

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

No studies have tested THN391 for the prevention of dementia or age-related cognitive decline.

Vascular pathology correlates with cognitive deficits (Gorelick et al., 2011; Toledo et al., 2013). Fibrinogen is undetectable in the healthy brain, but it leaks into the brain upon vascular breakdown or blood-brain barrier disruption.

In a meta-analysis of 2 observational studies (Rotterdam Study and Caerphilly Study), higher blood fibrinogen levels were associated with higher all-cause dementia (Darweesh et al., 2018). In the Rotterdam Study that included 2,835 people, those with higher levels of fibrinogen had an increased risk of dementia (Hazard Ratio [HR] per SD increase was 1.26; 95% CI, 1.11-1.44)(van Oijen et al., 2005). For Alzheimer's disease, the adjusted HR was 1.25 (95% CI, 1.04 to 1.49) and for vascular dementia the adjusted HR was 1.76 (95% CI, 1.34 to 2.30). In the Caerphilly study that included 865 men, higher fibrinogen levels were associated with higher risk of vascular dementia (HR=1.68; 95% CI, 1.02 to 2.76) after adjustment for age and risk factors (Gallacher et al., 2010). Higher factor VIII (HR=1.79; 95% CI,

1.09 to 3.00) and plasminogen activator inhibitor 1 (HR= 3.13; 95% CI, 1.73 to 5.70) were also associated with higher risk of vascular dementia. When all 3 hemostatic variables were high, the HR for vascular dementia was 2.97.

In an observational study of 185 elderly people with mild cognitive impairment, those with hyperfibrinogenemia (plasma fibrinogen over 3.0 g/L) at baseline had greater cognitive decline (measured by MMSE) during the 2-year follow-up than people with normal fibrinogen levels (-5.4 ± 5.4 vs. -3.5 ± 4.5; p<0.05)(Xu et al., 2008). Higher plasma fibrinogen level was associated with greater cognitive decline (R=0.17; p<0.05). People with hyperfibrinogenemia had an increased risk for dementia and vascular dementia compared with people with normal levels of plasma fibrinogen (p<0.05). There was a trend for an association between hyperfibrinogenemia and Alzheimer's disease (p=0.061).

Human research to suggest benefits to patients with dementia:

No studies have tested THN391 in people with dementia as of February 2024.

Vascular pathology is common in people with Alzheimer's disease and other dementias (Gorelick et al., 2011; Toledo et al., 2013). Fibrinogen leaks into the brain upon vascular breakdown or blood-brain barrier disruption and is often present in the brains of patients with Alzheimer's disease (Ryu and McLarnon, 2009; Viggars et al., 2011). In neurological diseases, fibrinolysis is impaired due to upregulation of plasmin inhibitors (e.g., plasminogen activator inhibitor 1), or due to the presence of Aβ (Cortes-Canteli et al., 2020). Aβ interacts with fibrinogen and coagulation factor XII, which can further increase fibrin deposition, clotting, and proinflammatory molecules, impairing tissue repair.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

The blood-brain barrier is a cellular barrier that tightly regulates the movement of molecules and cells between the blood and the brain (Daneman and Prat, 2015). It allows diffusion of oxygen, glucose, and other molecules critical for brain function, while restricting passage of pathogens, immune cells, and peripheral immune factors. Blood-brain barrier breakdown is associated with neurological diseases, including Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, epilepsy, and others, but also with normal aging. Blood-brain barrier disruption is also common in traumatic brain injuries. The amount of blood proteins in the cerebral spinal fluid (CSF) can indicate blood-brain barrier breakdown (Kantor et al., 2023). The CSF/plasma fibrinogen quotient is thought to be a marker for blood-brain barrier disruption.

Clinical data: At the 2023 Clinical Trials in Alzheimer's Disease (Boston, MA, Oct 2023), interim results from a phase I trial of THN391 was presented in a poster (Kantor et al., CTAD 2023 poster). The phase I study is designed to evaluate safety, tolerability, pharmacokinetics, and hematological outcomes and is not designed to evaluate neuroprotective benefits. THN391 has a 100-fold greater affinity for fibrin P2 than its parent murine monoclonal antibody, 5B8 (Kantor et al., CTAD 2023 poster).

Parent murine monoclonal antibody, 5B8: The murine monoclonal antibody 5B8 was developed against the cryptic fibrin epitope γ377-395, to selectively inhibit fibrin-induced inflammation and oxidative stress without interfering with clotting (Ryu et al., 2018). 5B8 selectively binds to fibrin, but not soluble fibrinogen, and does not interfere with activation of innate immune cells by ligands other than fibrin (e.g., LPS).

Models of Alzheimer's disease: In a mouse model of Alzheimer's disease (5xFAD mice), fibrin was detected in the brain as early as 3 months of age, and at 5 months of age, fibrin accumulation was abundant at sites of amyloid deposition surrounded by CD11b+ microglia (Ryu et al., 2018). In two mouse models of Alzheimer's disease (5xFAD mice and hAPP-J20 mice), in vivo imaging revealed dendritic spine elimination around fibrinogen deposits (Merlini et al., 2019). In wild-type mice, injection of human fibrinogen (at physiological blood concentrations) into the cortex led to dendrite and dendritic spine loss by day 3 compared to baseline and vehicle (artificial cerebrospinal fluid), suggesting that fibrinogen is sufficient to induce spine loss in the absence of amyloid pathology. Fibrinogen injection also caused microglia activation mediated by fibrinogen binding to CD11, suggesting that fibrinogen-induced spine loss is microglia-dependent. Fibrinogen-induced microglia activation via CD11b-CD18 promoted the generation and release of neurotoxic reactive oxygen species via NADPH oxidase, which is upregulated in neurodegenerative diseases.

In 5xFAD mice, systemic administration of 5B8 resulted in localization of 5B8 in fibrin-rich areas surrounding amyloid plaques, suggesting brain penetration and target engagement (Ryu et al., 2018). In 5xFAD mice, 5B8 treatment for 2 months started at 3.5 months of age reduced the loss of cholinergic neurons and microglia activation around plaques, without affecting amyloid plaques or the number of macrophages around plaques. Based on whole-genome microarray and gene ontology analysis of cortical gene expression in 5xFAD mice, 5B8 treatment suppressed five key pathways: complement pathway, antigen presentation, cytokine response, lysozyme, and reactive oxygen species.

Models of multiple sclerosis: Blood-brain barrier disruption and fibrin deposition occur early in multiple sclerosis, and in progressive multiple sclerosis, fibrin deposition in the cortex correlates with neuronal loss (Marik et al., 2007; Yates et al., 2017). In 3 mouse experimental autoimmune encephalomyelitis (EAE) models of multiple sclerosis, prophylactic 5B8 treatment reduced neurologic signs, reduced the mean maximum clinical score, and delayed the first day of onset compared to mice treated with IgG2b isotype control (Ryu et al., 2018). In a mouse model of encephalomyelitis induced by fibrinogen, intracerebroventricular 5B8 treatment inhibited microglial activation and chemokine gene expression. When 5B8 treatment was given therapeutically, severity of relapses and the percentage of paralyzed mice were decreased. Intraperitoneal injection of biotinylated 5B8 in mice with EAE showed that 5B8 localized to fibrin/fibrinogen-rich areas in the spinal cord, demonstrating target engagement, while not altering peripheral T cell responses, suggesting that 5B8 does not modulate peripheral adaptive immune responses.

In vitro studies: In bone marrow derived macrophages (BMDMs), 5B8 administration decreased fibrin-induced transcriptional activation of genes related to immune cell migration, adhesion, inflammatory responses, regulation of T cell proliferation, and chemotaxis (Ryu et al., 2018). 5B8 treatment in macrophages inhibited the fibrin-induced increase in protein expression of the Nox2 component gp91phox, phosphorylation of p40phox, and NADPH oxidase activity. In mouse and human macrophages, 5B8 treatment also inhibited fibrin-induced release of reactive oxygen species. Administration of 5B8 did not affect LPS-induced expression of proinflammatory genes, suggesting that the suppression of innate immune activation is selective for fibrin.

APOE4 interactions: It is not known yet whether THN391 has differential effects based on APOE4 genotype. In postmortem studies, brains from Alzheimer's cases homozygous for APOE4 had increased deposition of fibrin(ogen)(Hultman et al., 2013). APOE4 carriers also exhibit accelerated degeneration of pericytes that maintain the integrity of the blood-brain barrier compared to APOE3 carriers (Halliday et al., 2016). Therefore, theoretically, APOE4 carriers may benefit more from THN391 treatment than non-carriers.

Aging and related health concerns: No studies have tested THN391 in age-related diseases. In a mouse model of COVID-19, the murine 5B8 antibody reduced macrophage activation and oxidative stress in the lungs.

Types of evidence:

- Laboratory studies testing 5B8 in SARS-CoV2 models and human plasma

Beyond neurodegenerative diseases, fibrin deposition also occurs in other inflammatory pathologies such as rheumatoid arthritis, colitis, and Duchenne muscular dystrophy. Neither THN391 nor 5B8 have been tested in these conditions/models.

COVID-19, caused by SARS-CoV-2, is associated with increased blood clots, thrombotic events, and stroke. While these events are more common in severe cases of COVID-19, a case series study has reported that adults younger than 50 years old who had asymptomatic COVID-19 also experienced a higher incidence of acute ischemic stroke than people who did not have COVID-19 (Tu et al., 2021). Thromboinflammation, the interplay between blood clotting and inflammation, has been established as one of the major pathways driving COVID-19 pathology (reviewed in de Maistre et al., 2023). A preprint article reported that fibrin autoantibodies were abundant in 54 COVID-19 patients and persisted during the convalescent stage; and SARS-CoV-2 spike protein bound to fibrinogen and induced structurally abnormal blood clots with enhanced proinflammatory activity (Ryu et al., 2021 preprint, not peer-reviewed). In wild-type mice, SARS-CoV-2 spike glycoprotein activated macrophages and increased lung expression of the gp-91-phox subunit of NADPH oxidase, an enzyme involved in generating reactive oxygen species. Control virus proteins or the Env protein from HIV-1 did not have these effects. Mice deficient in fibrinogen (Fgα–/– mice) did not exhibit lung pathology following SARS-CoV-2 spike protein challenge. In wild-type mice, co-injection of SARS-CoV-2 spine protein and fibrinogen significantly increased fibrin-induced microglial activation, suggesting that the spike protein increases fibrin-associated inflammation. In wild-type mice given SARS-CoV-2 spike protein, treatment with the anti-fibrin antibody, 5B8, reduced macrophage activation and oxidative stress in the lungs compared to mice treated with isotype IgG2b control.

Incubation of SARS-CoV-2 spike protein in healthy donor plasma increased fibrin polymerization, with an altered fibrin clot structure consisting of thinner fibers with a rough appearance and an increased clot density (Ryu et al., 2021 preprint, not peer-reviewed). The SARS-CoV-2 spike protein bound to the γ364-395 peptide of fibrinogen, which encompasses the γ377-395 cryptic epitope, P2, which is the binding site to the CD11b/CD18 receptor (CR3) that activates innate immune responses. In bone marrow-

derived macrophages, the spike protein also increased fibrin-induced release of reactive oxygen species in a concentration-dependent manner, while the spike protein had no effect on reactive oxygen species in the absence of fibrin. In fibrin-treated bone marrow-derived macrophages, administration of 5B8 rescued the proinflammatory effects induced by the spike protein.

Safety: A phase I study in healthy subjects is ongoing, as of October 2023. Based on interim findings, THN391 is well-tolerated with no serious adverse events. Detailed results have not been published in a peer-reviewed journal.

Types of evidence:

- 1 phase I trial of THN391
- 1 review

Data on THN391: Based on interim results from a phase I trial of THN391 that were presented at the 2023 Clinical Trials in Alzheimer's Disease (Boston, MA, Oct 2023), THN391 was well-tolerated and no serious adverse events, infusion reactions, or hypersensitivity events were observed (Kantor et al., CTAD 2023 poster). The phase I trial evaluating THN391 consists of single- and multiple-ascending dose studies in healthy volunteers to assess its safety and tolerability, as well as pharmacokinetic and immunogenic properties (Therini Bio press release, Oct 24, 2023). At the time, the first 3 cohorts of 8 subjects each had received a single dose of 0.3 mg/kg, 1.0 mg/kg, and 3.0 mg/kg THN391. No drug related adverse events were reported and the therapy was found to be well-tolerated. Dose-proportional Cmax levels were observed. At the single 0.3 mg/kg and 1.0 mg/kg dose, the half-life was approximately 50 days. Additional single-ascending dose study at 10 mg/kg THN391 and multiple-ascending dose study at 3.0 mg/kg had been started.

In preclinical and nonclinical safety studies, THN391 was safe and well-tolerated, with no effects on coagulation (reviewed in Kantor et al., 2023). No central nervous system adverse effects were observed in rats or monkeys with THN391, and no adverse effects were observed on cardiovascular and respiratory systems in monkeys with intravenous doses up to 100 mg/kg. There were also no signs of global immunosuppression or increased infection following 2 months of THN391 dosing in 4-week rat and nonhuman primate Good Laboratory Practices toxicology studies (up to 100 mg/kg). THN391 did not bind fibrinogen and did not interfere with coagulation ex vivo in activated partial thromboplastin time and thromboelastography assessments. (Kantor et al., CTAD 2023 poster).

Data on the parent murine monoclonal antibody, 5B8: 5B8 selectively binds to fibrin, but not soluble fibrinogen, and inhibits binding of fibrin to complement receptor 3 (CR3) without interfering with polymerization time of fibrinogen to fibrin, *in vivo* murine plasma clotting time, or partial thromboplastin time (aPTT) in human plasma (Ryu et al., 2018). Mice treated with 5B8 for periods up to 2 months did not develop any spontaneous bleeding.

Drug interactions: Drug interactions have not been studied to date.

Sources and dosing:

THN391 is under development by Therini Bio, Inc. for the treatment of dementia and other inflammatory neurodegenerative diseases such as multiple sclerosis. Dosing has not been established. Phase I single-ascending dose (0.3, 1.0, 3.0, and 10 mg/kg, i.v.) and multiple-ascending dose studies (3 mg/kg, every 2 weeks and every 4 weeks, i.v.) are ongoing (Kantor et al., CTAD 2023 poster).

Research underway:

No clinical trials testing THN-391 are registered on ClinicalTrials.gov. Based on a poster presentation at the 2023 Clinical Trials in Alzheimer's Disease (Boston, MA, Oct 2023), interim results from a phase I trial of THN391 was presented (Kantor et al., CTAD 2023 poster). Single-ascending dose studies of 0.3, 1.0, and 3.0 mg/kg doses had been completed, with the 10 mg/kg dose study ongoing at the time of the presentation. The multiple-ascending dose studies at the 3 mg/kg 2x weekly dose was ongoing at the time. Also, a 6-month Good Laboratory Practice chronic toxicity study in nonhuman primates was also ongoing.

Therini Bio Inc. is also planning a phase 1b trial in an enriched patient population of mild to moderate Alzheimer's disease with inclusion criteria based on MMSE, plasma p-tau, and structural MRI (Kantor et al., CTAD 2023 poster). They aim to enroll 48 patients, with a 2:1 randomization and a 6-month treatment duration, with up to a 6-month follow-up. Biomarker outcomes include Aβ40, Aβ42, tau, neurodegeneration markers (Nfl, VILIP-1), microglial markers (sTREM2, sCSF1R, IL1RN, SPP1, osteopontin), astrocyte markers (YKL-40, GFAP, S100B), and cytokine/chemokine panels.

Search terms:

Pubmed, Google: THN-391, 58B, fibrinogen, fibrin

Websites visited for THN-391:

- Clinicaltrials.gov (0)
- NIH RePORTER (0)
- Drugs.com (0)
- WebMD.com (0)