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.

Anti-C1q

Evidence Summary

Inhibiting C1q may slow complement-mediated synaptic/neuronal loss in neurodegenerative diseases as a treatment. It is not well-suited for prevention. Clinically tested inhibitors have been well-tolerated.

Neuroprotective Benefit: C1q could be protective by promoting clearance of cell debris, or harmful by promoting excessive synapse elimination and neurodegeneration. Anti-C1q could be protective in a high inflammation, high complement environment.

Aging and related health concerns: High C1q levels are associated with age-related muscle loss, vascular remodeling, glaucoma, age-related macular degeneration, liver disease, and poor cancer prognosis. Impact of inhibition may be context dependent.

Safety: Systemic C1q antibodies may increase the risk for autoimmunity and infection in predisposed individuals. They have shown a favorable short term safety profile. Local infusion/injection site reactions have been the most common adverse event in clinical trials.

Availability: In clinical trials	**Dose**: Not established ANX005 and ANX105 are administered i.v. ANX007 is administered via intravitreal injection. ANX009 is administered s.c. ANX1502 is administered orally	**Chemical formula:** N/A **MW**: N/A
Half-life: ANX005: 50-106 hours (50 -200 mg/kg) based on preclinical studies ANX007: ~ three days (1-5 mg dose) based on preclinical studies	**BBB**: Minimal penetrance 0.1-0.2% (similar to other antibodies)	
Clinical trials: ANX005 (n=116 to date): Phase 1 trials in healthy volunteers and Guillain Barré Syndrome. Phase 2 trials in ALS, HD, and hemolytic anemia. ANX007: Phase 1 trials in glaucoma (n=26), and Phase 2 trial in geographic atrophy (n=270). ANX009: Phase 1 trial in healthy volunteers (n=48). ANX1502: Phase 1 trial in healthy volunteers (n=84).	**Observational studies**: High serum C1q has been associated with some age-related diseases. High brain/CSF C1q has been associated with neurodegenerative diseases.	

What is it?

C1q is a glycoprotein containing 18 polypeptide chains composed of 6 repeats of 3 polypeptides (C1qA, C1qB, C1qC) [1]. It is **the initiating protein of the classical complement cascade**. It is involved in the regulation of multiple cellular functions, including innate and adaptive immunity, through a variety of receptors. It has both complement cascade dependent and complement independent functions. C1q is synthesized and secreted locally, so its function in a given tissue is dependent on the microenvironment, such as the receptor milieu within a given tissue. Tissue damage in the context of autoimmune disease and some neurodegenerative diseases is associated with excessive levels of complement activation. Anti-C1q antibodies are being developed for the treatment of autoimmune and neurodegenerative diseases [2]. Annexon Biosciences has developed several anti-C1q antibodies that have been formulated for different modes of administration and are being targeted towards different disease indications.

ANX005 is a humanized immunoglobulin G4 recombinant monoclonal antibody targeted against C1q that is administered via intravenous infusion [3]. It has been tested in Phase 1 clinical trials in healthy volunteers, and in patients with Guillain Barré Syndrome. It has been tested in Phase 2 clinical trials in patients with warm autoimmune hemolytic anemia, and Huntington's disease, and is currently being tested in a Phase 2 trial in patients with amyotrophic lateral sclerosis.
ANX105 is a next-generation full length monoclonal antibody targeting C1q administered via intravenous infusion that has been designed to have better dosing properties relative to ANX005 (Corporate Presentation). It is currently being tested in a Phase 1 trial in healthy volunteers.

ANX007 is a high affinity monoclonal antibody antigen-binding fragment (Fab) that specifically recognizes the substrate-binding head groups of C1q [4]. It has been formulated for ocular diseases, and is administered locally to the eye via intravitreal injection. It has been tested in Phase 1 trials in patients with primary open angle glaucoma, and is currently being tested in a Phase 2 trial in patients with geographic atrophy secondary to age-related macular degeneration.

ANX009 is an antigen-binding fragment (Fab) of a humanized antibody against C1q that has been formulated for subcutaneous administration [5]. It has been tested in a Phase 1 trial in healthy volunteers. It is being developed for vascular antibody-mediated autoimmune diseases, and is currently being tested in a Phase 1 trial in patients with lupus nephritis.

ANX1502 is a high-affinity, selective (200 to 50,000-fold), small-molecule inhibitor of C1q (IC_{95}=100 nM) that is currently being tested in a Phase 1 trial in healthy volunteers (Corporate Presentation). It is being developed for autoimmune conditions, including multifocal motor neuropathy.

Neuroprotective Benefit: C1q could be protective by promoting clearance of cell debris, or harmful by promoting excessive synapse elimination and neurodegeneration. Anti-C1q could be protective in a high inflammation, high complement environment.

Types of evidence:

- 1 meta-analysis of gene association studies regarding variants in CR1 and AD
- 1 genetic association study regarding complement CR1 variants with AD
- 1 systematic review of studies examining complement proteins in AD
- 1 meta-analysis of exosome-based biomarker studies in AD

- 23 observational studies (complement expression in brain or biofluids in neurodegenerative disease)
- Numerous laboratory studies

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

No studies have directly tested whether anti-C1q therapy could prevent dementia or cognitive decline. However, observational studies suggest associations between C1q levels and brain aging. Levels of C1q have been found to increase in the human brain by as much as 300-fold in the course of normal aging [6]. Based on postmortem tissue analysis, it is localized near synapses, and is particularly enriched in regions known to be vulnerable to neurodegeneration, including the hippocampus**.**

A single nucleotide polymorphism (SNP) in the complement receptor CR1 gene (rs4844609 - Ser1610Thr) is associated with episodic memory decline (β (SE) = −0.034 (0.012), P = 0.005) [7]. This SNP alters the conformation of CR1 in a manner that may reduce its binding affinity for C1q, thereby reducing the efficiency of clearance of plaques/cellular debris. Indeed, it is also associated with increased susceptibility for Alzheimer's disease (AD) (Odds ratio [OR]: 1.40, 95% CI 1.02 to 1.94) and increased plaque burden (β (SE) = 0.244 (0.106), P = 0.021). Other gene variants which impact the structure and function of CR1 have also been associated with risk for AD. A meta-analysis including 13 case-control studies in Caucasian populations (n=10,704 cases; 12,360 controls) found that carriers of the A allele in the rs6656401A/G SNP showed increased risk for AD relative to G carriers (OR: 1.23, 95% CI 1.16 to 1.30) [8]. A meta-analysis of five case-control studies (n= 4740 cases; 8495 controls) found that for the rs3818361T/C SNP, having the T allele was associated with higher AD risk (OR: 1.21, 95% CI 1.13 to 1.31) [8]. This suggests that C1q dysregulation, where there is a loss of its protective role in promoting clearance of cellular debris, and increase in its destructive synapse eliminating activity, is involved in age-related cognitive decline, and may be related to changes in the inflammatory milieu.

Human research to suggest benefits to patients with dementia**:** None

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

C1q appears to have both neuroprotective and neurodegeneration promoting properties in the CNS depending on conditions in the microenvironment. C1q has both complement-dependent and complement-independent functions, and the presence or absence of interacting partners necessary for

mediating these processes can influence whether beneficial or deleterious processes dominate. **In the context of aging, there appears to be a shift toward conditions that promote the neurodegenerative activity of C1q.**

Age-related cognitive decline: C1Q LEVELS ARE ELEVATED IN THE AGING BRAIN
Similar to humans, levels of C1q increase in the mouse brain during aging. This age-related increase in hippocampal C1q was associated with age-related cognitive decline, and this decline could be prevented in absence of C1q [6]. Hippocampal increases in C1q were found to be higher in aged female mice, though it was significantly increased in both sexes [9]. Notably, females also had greater increases in inflammatory microglial-associated genes during aging, suggesting that the high inflammation neuro-environment could promote pathological neurodegenerative processes, which contributes to an increased risk for dementia. Changes to C1q levels in the aging brain were also analyzed in rhesus macaques and Sprague-Dawley rats. C1q levels were increased with age (>age 20) in the macaque dorsal lateral prefrontal cortex, such that the elevated levels correlated with the period of synaptic (dendritic spine) loss in this brain region [10]. C1q was preferentially localized near synaptic spines and near abnormal mitochondria in dendrites. Calcium overload from the dysfunctional mitochondria may be a driver of C1q release by inflammatory glia. A similar age-related pattern of synaptic C1q deposition was also seen in the rat prefrontal cortex. The elevations in C1q were associated with local accumulations of reactive glia.

Alzheimer's disease: POTENTIAL BENEFIT FOR TREATMENT (Preclinical)
Circulating C1q levels have been shown to be elevated in the context of acute neuronal injury [11]. Biomarker studies have also shown a general increase in C1q levels in the context of AD, though levels can be impacted based on age, stage of disease, and type of biofluid. Due to the expression of C1q throughout the body, peripheral blood-derived levels of C1q are not a clear representation of C1q activity in the brain, whereas CSF measures may be more informative. A systematic review and meta-analysis assessing changes to complement proteins in AD found that CSF C1q levels were not significantly different, however, two of the four included studies were confounded by age differences between the AD and control populations [12]. The disease-associated change in C1q levels has been more apparent in studies examining extracellular vesicles. A meta-analysis of 19 studies assessing the utility of blood-based exosome biomarkers in AD found that levels of C1q were significantly increased in astrocyte-derived exosomes in AD patients relative to healthy controls (SMD: 3.16, 95% CI 2.57 to 3.76; $P < 0.001$) [13]. A separate multi-cohort study including a total of 166 AD patients and 90 controls found that the levels of C1q in CSF-derived extracellular vesicles was increased in AD relative to controls,

especially during the MCI stage [14]. C1q levels were higher in ApoE4 carriers relative to non-carriers. Additionally, levels of C1q in CSF-derived extracellular vesicles were associated with Mini-mental status exam (MMSE) scores in MCI/AD patients, but levels of total CSF C1q were not associated with MMSE. A study examining complement proteins in astrocyte-derived exosomes (n=60) found that levels of activated complement cascade proteins including C1q, C4b, C5b, C3b, and C5b-C9 were significantly elevated in patients with MCI who converted to AD relative to those who were stable over the course of 36 months [15]. Additionally, converters had lower levels of inhibitory complement proteins. The association between extracellular vesicle derived C1q and cognitive decline is likely a reflection of the removal of C1q-tagged synapses by phagocytic glia, such that higher levels of synaptic loss drive the association with cognitive decline. Elevated C1q may also contribute to AD via the recruitment of neutrophils. Neutrophil extracellular traps (NETs) are designed to trap and kill pathogens, but have been detected near Aβ plaques and may contribute to inflammation and tissue injury. C1q promotes NET accumulation, and plasma/serum levels of NETs were also found to be elevated in AD patients [16]. The microRNA-132 is enriched in the brain, and has been found to be decreased in AD [17]. miR-132 contains a binding site for C1q mRNA, suggesting that its loss may contribute to AD progression via the upregulation of C1q levels [18]. In postmortem brain tissue (frontal cortex), C1q levels were associated with levels of pro-inflammatory (CHI3L1+) astrocytes and (Iba1+) microglia during early stages of AD [19]. Together these studies suggest that elevated C1q levels in early AD are associated with a CNS environment characterized by excessive synapse loss driven by dysfunctional glia.

In a healthy CNS, C1q may play a protective role in preventing AD-associated pathology, however, this **protective role appears to be largely dependent on the functional state of astrocytes, particularly their phagocytic capacity.** The effect of C1q is partially dependent on ApoE, and the increased burden of AD-related pathology associated with the CR1 susceptibility allele was found to be dependent on an interaction with ApoE4.

C1q has been found to be upregulated in the context of neuronal injury and have neuroprotective effects *in vitro* by activating p-CREB and AP-1, which in turn activates LRP1B and GPR6 [20]. In young (2 month) 3xTg mice before the onset of symptoms, this neuroprotective pathway was found to be active. Under these conditions, C1q levels were increased, but other downstream complement components were not elevated. Meanwhile, complement was upregulated in older symptomatic mice when neuroprotection was lost. Neuron-associated C1q was also found to be elevated in the hippocampus and frontal cortex of a woman (age 47, ApoE2/4) with preclinical AD, which may have been a transient

neuroprotective response to injury [21]. In later stages, C1q is localized to both plaques and neurons [21; 22]

Part of this neuroprotective response involves the clearance of Aβ and other cellular debris [23]. Astrocytes release both C1q and ApoE. **The interaction between C1q and ApoE is important for controlling astrocyte function and regulating inflammation.** Astrocytes are involved in eliminating damaged or unwanted synapses through C1q dependent phagocytosis [24]. ApoE2 expressing astrocytes have efficient phagocytic capacity, whereas ApoE4 astrocytes have reduced phagocytic capacity [25]. The phagocytic capacity of astrocytes was also found to be reduced in the context of aging, and in the 5XFAD model relative to wildtype mice [23]. This discrepancy is driven, in part, by a shift from less A2-like pro-phagocytic astrocytes to more A1-like pro-inflammatory astrocytes [26]. As a result of reduced clearance, there is a defect in synaptic turnover and debris removal, which can lead to an accumulation of C1q [25]. This defect in clearance can then promote the infiltration and activation of microglia, leading to inflammation and a loss of C1q tagged synapses and neurons.

Impaired astrocytic uptake of extracellular glutamate following synaptic transmission may also facilitate the accumulation of C1q on synapses. The loss of astrocyte glutamate transporter (GLT1) protein expression and reduced glutamate uptake are associated with cognitive decline in AD [27]. In rats, astrocytic activation following exposure to Aβ, led to a reduction in GLT1, similar to what is seen in AD patients [28]. The reduction in glutamate uptake led to elevated levels of glutamate following synaptic transmission, such that glutamate could now activate both synaptic receptors, as well as extrasynaptic receptors, known as metabolic glutamate receptors (mGluRs). Several studies have shown that activation of the mGluRs can facilitate the accumulation C1q at synapses, and subsequent synaptic loss. Treatment of the Aβ-exposed rats with the antibiotic ceftriaxone, which can upregulate GLT1 expression, reduced C1q synaptic tagging and loss [28]. Similarly, treatment of AD mouse models (APPswe/PS1ΔE9 and AppNL-G-F/hMapt double knock-in) with a silent allosteric mGluR5 modulator which blocks the Aβ-induced increase in mGluR5 activity also reduced the accumulation of C1q to synapses and associated synaptic loss in these animals [29].

The transition from a neuroprotective role for C1q to a neurodegenerative role appears to coincide with the upregulation of other complement components in the brain, such that its functions go from being primarily complement-independent to complement-dependent. The CNS is particularly vulnerable to complement mediated toxicity because it has a very low level of complement regulatory/inhibitory proteins relative to other organ systems. Expression of complement proteins (C1 through C5) has been

found to increase in the mouse brain during aging; similar increases have also been found in the human brain, especially C4 levels [30]. Complement accumulation is increased in AD mouse models [31], and in the human AD brain there is specific increase in proteins late in the complement cascade (C5b-9) that make up the membrane attack complex which facilitates complement-mediated cell death [30]. Astrocyte-derived exosomes from individuals that transitioned from preclinical to clinical AD contained higher levels of complement effectors and inflammatory proteins after the transition [26]. The use of an anti-C1q monoclonal antibody ANX-M1 (from Annexon) prevented Aβ induced synapse loss in wildtype mice, and APP/PS1 transgenic mice lacking C3 did not experience deleterious microglial-driven synapse loss [32]. The impact of targeting the complement pathway likely depends on the stage of disease, such that during early/preclinical periods the complement cascade could potentially have neuroprotective effects by enhancing debris clearance. For example, treatment with EP67, an oral C5a receptor agonist was found to reduce Aβ plaque load by enhancing the phagocytic capacity of glia in the 5XFAD mouse model, when administered very early in the disease course, at three months of age [33].
These studies suggest that blocking the classical complement cascade in the brain could potentially slow the progression of neurodegeneration in AD. While blocking the cascade at a late stage, such as with a C5 inhibitor, would be expected to be most effective for eliminating complement-mediated cytotoxicity, it would also pose a higher risk for increasing susceptibility to brain infections.

The CNS increase in late complement components with aging and in AD may be related to changes in neurovascular integrity influenced by the C1q-ApoE interaction. C1q can bind to oxidized lipids and promote their uptake by macrophages [34]. These lipids can also serve as a surface for complement activation. Therefore, the **C1q binding to lipids accumulated at blood-CNS barriers can attract immune cells, drive their infiltration, and lead to breakdown of these neurovascular barriers** [35]. Influx of Ig across these weakened barriers can promote complement expression and signaling. Some of the Ig may bind to CNS proteins and activate complement-dependent cytotoxicity. The binding of ApoE to C1q can prevent binding to the oxidized lipids, and associated complement activation and inflammation [35], though the impact of this complex is context-dependent, and can vary based on the modification state of ApoE [36]. ApoE secretion by astrocytes was found to decrease during aging in mice, which may contribute to the age-related decline in neurovascular integrity [37]. Long-term aerobic exercise prevented deterioration of neurovascular structures during aging in mice by preventing the age-related decline in ApoE and increase in C1q. This suggests that therapies which reduce C1q levels may promote healthy aging.

Frontotemporal dementia: C1Q IS ELEVATED IN THE CNS WITH DIESASE PROGRESSION

Biomarker studies indicate that C1q is upregulated in the context of tauopathies, including Frontotemporal dementia (FTD), which may coincide with cognitive deficits. A study of 275 pre-symptomatic carriers, 127 symptomatic carriers, and 247 non-carriers of FTD-associated mutations in GRN, C9orf72 or MAPT from the GENFI cohort assessed the trajectory of CSF biomarkers in the context of conversion [38]. Of the examined biomarkers, CSF NPTX2 was the first to become abnormal (decrease), whereas CSF C1q and C3b did not become abnormal (increase) until later stages of disease. This pattern may arise because the increase in complement occurs downstream of the loss of NPTX2 [39]. Associations were seen between levels of these biomarkers and cognitive scores on the MMSE and CDR® + NACC FTLD-SB in mutation carriers [38]. A separate study from the GENFI cohort (n=224) found that CSF C1q and C3b levels were correlated with each other and upregulated in symptomatic carriers (C1q: median 362 ng/ml; interquartile range 284 to 481) relative to pre-symptomatic carriers (median 256 ng/ml; interquartile range 199 to 337), and the effect was most prominent in C9orf72 mutation carriers [40]. C1q levels increased in conjunction with neuronal damage based on associations with higher NFL levels and lower frontal lobe volume. CSF (n=224) and plasma (n=431) levels of C1q were not correlated in this cohort, suggesting that they are derived from different sources. Similarly, CSF C1q levels were found to be elevated in another group of symptomatic FTD-mutation carriers (n=21), relative to pre-symptomatic carriers (n=40) and non-carriers (n=22), and correlated with C3b levels [39]. Notably, levels of CSF C1q complexed with NPTX2 was found to be decreased in symptomatic carriers [39]. The loss of the association between NPTX2 and C1q may facilitate the activation of the complement pathway.

C1q has been shown to interact with neuronal pentraxins at excitatory synapses [41]. Soluble complement regulatory proteins C4BP and factor H also bind to limit complement activation. The loss of NPTX2 is associated with increased C1q-mediated synaptic elimination. Treatment with a C1q-blocking antibody was able to increase synaptic density in NPTX2 knockout mice, while the overexpression of NPTX2 was able to reduce synaptic loss in TauP301S mice [39]. This suggests that the loss of NPTX2 may be driving the activation of the complement pathway and complement-mediated synapse loss. The increase in C1q and complement activity appears to be a common feature of both familial and sporadic FTD. The upregulation of neuronal adenosine A2A receptor signaling was correlated with an increase in C1q in postmortem temporal cortex tissue from an FTD patient with the MAPT P301L mutation [42]. A similar association was seen in postmortem frontal cortex tissue from patients with sporadic FTD (Corticobasal degeneration and Pick's disease). In THY-Tau22 tauopathy mice, the overexpression of the A2A receptor in neurons resulted in the upregulation of C1q and the loss of glutamatergic synapses [42].

Notably, the increase in C1q in the tau mice around nine months of age correlated with the time that the mice started to show significant memory deficits.

Progranulin deficiency is associated with age-related increases in microglial activation, complement production, and microglia mediated synaptic loss. In brain tissue from FTD patients with progranulin mutations, microglial density was increased in association with C1q deposits [43]. Furthermore, levels of complement proteins C1q and C3 increased as cognitive function declined, based on the MMSE. In the context of progranulin deficiency in mice, loss of C1q prevents synaptic loss and neurodegenerative phenotypes [43]. The neuronal damage following microglial driven phagocytosis of C1q tagged synapses exacerbated tau pathology in mouse models [44]. These studies suggest that blocking C1q could potentially slow the progression of neuronal and synaptic loss stemming from altered neuronal activity related to tau pathology.

Parkinson's disease: C1Q LEVELS ARE ELEVATED IN SYNUCLEOPATHIES
Complement pathway activation may participate in alpha-synuclein-related toxicity in the context of synucleinopathies. In alpha-synuclein expressing SH-SY5Y neuroblastoma cells, C1q was a mediator of complement-dependent toxicity [45]. In postmortem tissue, there was a trend for higher levels of C1q in the putamen of patients with multiple systems atrophy (n=4), a region with disease pathology, relative to controls (n=4), while there was no difference in C1q levels between the groups in the visual cortex, a brain region without disease pathology [45].

Progressive multiple sclerosis: C1Q LEVELS ARE ELEVATED IN GRAY MATTER
Neurodegeneration is a prominent feature of progressive multiple sclerosis (MS). The drivers of gray matter degeneration appear to be distinct from the T cell-mediated bouts of demyelination in the white matter. In an analysis of postmortem brain tissue from patients with progressive MS (n=21) and matched controls (n=10), neuronal loss in the thalamus was correlated with disease duration and age of death [46]. There was evidence for increased classical complement pathway activation in the thalamus, based on increased levels of C1q, C4d, Bb and C3b, whereas levels of the complement C1 inhibitor remained low. A separate study examining postmortem brain tissue found evidence for complement activation around the CSF-brain barriers, including the leptomeninges, subpial, and within and around vessels of the cortical grey matter [47]. The level of C1q was associated with the degree of inflammatory cell infiltration along the barriers, while the degree of gray matter demyelination was associated with the level of activated complement (C4d, Bb, and C3b). These studies suggest that excessive complement activation may be a driver of gray matter damage in progressive MS.

Traumatic brain injury: SERUM C1Q LEVELS ELEVATED AFTER BRAIN INJURY

Serum C1q levels were found to be elevated in patients with traumatic brain injury (TBI) (n=188) (median 223.9 mg/L) relative to controls (n=188) (median 75.4 mg/L) [11]. The increase in serum C1q was associated with the severity of neurological injury based on Glasgow coma scale (GCS) score, Rotterdam CT classification, and Glasgow outcome scale-extended (GOSE) score. Additionally, serum C1q served as a prognostic factor for outcome, with levels above 345.5 mg/L serving as a predictor for six-month poor outcome (OR: 4.922, 95% CI 1.552 to 15.606; P = 0.017). Since C1q levels have been shown to be elevated in the brain around injury sites, the increase in serum C1q is thought to be at least partially a reflection of increased C1q in the CNS following brain injury.

Amyotrophic lateral sclerosis: POTENTIAL BENEFIT

Evidence of classical complement pathway activation has been detected in the CNS in patients with amyotrophic lateral sclerosis (ALS). This includes increases in CSF levels of C4 [48], and elevated levels of C1q and C4, which was accompanied by an increase in activated innate and adaptive immune cells, in spinal cord and motor cortex tissue from ALS patients [49]. C1q was found to be deposited on the motor endplates from intercostal muscle biopsies from ALS patients, which was co-localized with neurofilament light (NFL) and the terminal complement complex or membrane attack complex (MAC) [50]. However, the preclinical evidence regarding whether C1q and complement meaningfully contribute to disease progression has been mixed. One study found that deletion of C1q did not impact glial activation, motor neuron loss, or disease progression in the SOD^{G37R} mouse model [51]. There was also evidence of increased presynaptic terminal loss and a modest acceleration of disease course in male SOD^{G37R} mice lacking C1q. Meanwhile, a separate study found that knocking out IL-1α, TNFα and C1qa slowed disease progression and extended lifespan in $SOD1^{G93A}$ mice [52]. The protective effect was mediated by the lack of C3+ reactive (A1) astrocytes in these mice. These studies suggest that while C1q and complement activation likely play a role in ALS, targeting C1q alone may be insufficient to impact disease progression in a clinically meaningful way.

ANX005 is currently being tested in an open-label Phase 2a clinical trial in patients with ALS (n=24) (NCT04569435). ANX005 is being administered via i.v. infusion on days one and five (or six), followed by maintenance doses every two weeks for up to 22 weeks. Preliminary data from eight patients found a trend toward a reduction in plasma NFL levels during 12 weeks of treatment, with a rebound during the off-treatment period (Corporate Presentation). Similarly, all patients showed stabilization or improvement on the ALSFRS-R C, a measure of disability progression during the treatment period, but

declined again following treatment cessation. The two participants who maintained treatment for the full 24 weeks did not experience functional declines over this period. The full data is expected to be read out later in 2023.

Huntington's disease: POTENTIAL BENEFIT

Complement components, including C1q, were found to be elevated in the striatum of patients with Huntington's disease (HD) relative to controls [53]. The mRNA levels of complement components were two to five times higher in HD patients, and the activated complement components were primarily expressed by reactive glia. Administration of 3-nitropropionic acid to the rodent striatum results in a condition that mimics HD. In rats, administration of 3-nitropropionic acid was shown to induce the production of neurotoxic A1 astrocytes, which are characterized by the expression of IL-1α, TNFα and C1q, in conjunction with the activation of complement C3 [54].

ANX005 was tested in an open-label Phase 2 clinical trial in patients with, or at risk for early manifest HD based on a CAG-Age-Product (CAP) score >400 and a Unified Huntington's Disease Rating Scale (UHDRS) independency score ≥80% (NCT04514367). ANX005 was administered via i.v. infusion on days one and five (or six), followed by maintenance doses every two weeks for up to 22 weeks with a three-month follow-up. Twenty-eight patients were enrolled and 23 completed the study. CSF levels of C3, a marker of complement downstream of C1q, were reduced with ANX005 treatment, and remained low during the off-treatment period (Corporate Presentation). The degree of improvement on inflammatory and functional measures was stratified by the baseline level of complement activation, based on the ratio of C4a/C4. Patients with high baseline levels of activated complement showed a reduction in CSF levels of YKL-40, a measure of inflammatory activated glia, which waned following treatment cessation. Additionally, this subset of patients also showed a trend toward improvement on the composite UHDRS, with 75% of participants with high baseline complement showing a reduction in disease progression at week 24, compared to 36% of participants with low baseline complement (Press release). Based on the results of this study, a pivotal Phase 2/3 study is planned for 2023 in this population.

APOE4 interactions:

ApoE4 carriers would be expected to preferentially benefit from anti-C1q therapy, because the presence of ApoE4 reduces the phagocytic capacity of astrocytes and appears to mitigate the neuroprotective benefits of C1q, while potentiating its deleterious effects [25]. While all ApoE isoforms were found to have similar binding affinities to C1q, the increased lipid load associated with ApoE4 status may exceed the levels of the protective ApoE-C1q complex, especially as ApoE levels decline, and increase the vulnerability of neurovascular damage [35].

Aging and related health concerns: High C1q levels are associated with age-related muscle loss, vascular remodeling, glaucoma, age-related macular degeneration, liver disease, and poor cancer prognosis. Impact of inhibition may be context dependent.

Types of evidence:

- 1 systematic review examining complement levels in the context of exercise
- 19 observational studies (serum/plasma/tissue C1q levels in association with age-related muscle loss, diabetes and atherosclerosis, obesity, coronary artery disease, aging, stroke, exercise, age-related macular degeneration, glaucoma, cancer, and liver disease)
- Numerous laboratory studies

Age-related muscle loss: HIGH C1Q LEVELS ASSOCIATED WITH MUSCLE LOSS

Levels of circulating C1q have been found to increase with age. In a cross-sectional study including subjects from 20 to 81 years of age (n=131), serum C1q levels were inversely correlated with age, thigh cross-sectional area, muscle mass, and muscle (knee) power, and were positively correlated with pro-inflammatory markers (IL-6, TNF) [55]. Twelve weeks of (knee) resistance training reduced age-associated increases in C1q while increasing thigh cross-sectional area ($r = -0.703$; $P < 0.01$) in older men (n=11; age 60-81 years). A systematic review examining the relationship between complement proteins and exercise from 77 studies including 10, 236 participants found that C1q levels were inversely correlated with muscle strength [56]. Strength training involves the induction of microtears to the muscle, which promote the activation of muscle stem cells during the repair phase. C1q is secreted by M2-like macrophages during the resolution/repair phase following muscle injury, which corresponds with the peak in C1q levels two to four days post muscle injury. C1q-mediated clearance of apoptotic muscle fibers may facilitate the process of muscle cell regeneration and growth. Endurance athletes show lower levels of circulating active complement components and inflammatory cytokines relative to athletes in low to moderate endurance sports [57]. However, a study in 79 elite athletes found that the effect was impacted by age, such that due to age-related increases, serum measures of complement components, such as C1q, were lower in young athletes (<30 years old) relative to older athletes [57]. Age related increases to C1q may also impact lean mass by impacting bone cell dynamics. A study in cell culture found that a modest increase in C1q at physiologically relevant levels could bias bone cell progenitors toward the production of bone-absorbing osteoclasts [58].

Preclinical studies in rodents suggest that the age-related increase in C1q may play an active role in inhibiting muscle repair and regeneration. In senescence accelerated SAMP1 aged mice (38 weeks old), 12 weeks of resistance training reduced circulating C1q levels which was associated with a reduction in

muscle fibrosis ($r = 0.640$, $P < 0.05$) and increased muscle mass ($r = -0.375$, $P < 0.05$) [59]. Pre-administration of recombinant C1q prevented these protective effects and suppressed muscle regeneration [59; 60]. Secreted C1q can bind to frizzled receptors which activates canonical Wnt signaling in satellite cells and fibroblasts, which in turn, promotes muscle fibrosis and inhibits regeneration [61]. This activity is independent of classical complement activation and instead involves C1q mediated, complement component 1s (C1s) dependent cleavage of the ectodomain of the Wnt co-receptor, LRP6.

These studies suggest that exercise protects against age-related muscle loss, in part, by reducing C1q. Therefore, anti-C1q therapy could potentially help protect against muscle loss and frailty with age. However, it is not clear what is driving the age-related increase in C1q or how it is reduced by exercise, so it is possible that other factors are required to see benefit.

Cardiovascular: C1Q IS DYSREGULATED IN CVD IN A CONTEXT-DEPENDENT MANNER

The longitudinal CODAM (Cohort on Diabetes and Atherosclerosis Maastricht) study (n=574, 60±7 years, 61% men) examined the seven-year incidence of cardiovascular disease (CVD) and found that cardiovascular health was associated with serum C1q levels in the middle tertile [62]. High or low C1q levels were associated with 2 to 2.5-fold higher risk [T_{low} vs T_{middle} odds ratio (OR): 2.38 (95% confidence interval, 1.14 to 4.95]; T_{high} vs T_{middle} OR: 1.96 (95% CI, 0.94 to 4.07)]. This suggests that there may be an optimal level of C1q with respect to CVD. C1q can play both beneficial and detrimental roles with respect to the cardiovascular system, depending on the context. C1q has both complement-dependent, and complement-independent functions. Its roles in the clearance of lipids and cell debris are expected to be beneficial, however, it can also drive vascular smooth muscle cell remodeling and complement-mediated inflammation. Therefore, the functional outcome of elevated C1q levels can vary in different cardiovascular disease conditions and stages. One variable which may impact the utility of C1q as a biomarker is the degree of systemic inflammation. At low levels of inflammation, C1q may be preferentially contributing to productive processes, while high systemic levels of inflammation could be indicative of complement overactivation. An observational study in 1,701 patients with acute coronary syndrome found that higher levels of serum C1q were associated with lower risk for major adverse cardiovascular events (MACE) (HR: 0.561, 95% CI 0.375 to 0.840), but only in patients with low levels of systemic inflammation, as defined by hs-CRP levels less than 2 mg/L [63].

Coronary artery disease (CAD): Men (n=159) with diabetes and suspected CAD who had low levels of serum C1q (≤179 ug/ml) were found to have a higher rate of all-cause mortality (65.4%) than those with high levels of serum C1q (46.8%) (adjusted hazard ratio [HR]: 0.66, 95% CI 0.52 to 0.84, P = 0.0006) [64].

C1q can bind and interact with a variety of different proteins and form protein-complexes. The levels of C1q bound in these complexes could be important for regulating its activity, thus altered complex formation could potentially play a role in age-related diseases. Adiponectin is an adipokine secreted by adipose tissue that exerts beneficial effects on insulin sensitivity and glucose homeostasis. One study (n=153) found that having a high ratio of C1q complexed with adiponectin relative to total adiponectin levels was associated with more severe coronary stenosis (OR: 2.09, 95% CI 1.12 to 3.91) [65]. Similar associations were found with respect to atherosclerosis and CAD risk [66]. High levels of adiponectin complexed C1q relative to total C1q was also associated with aging (r=0.09, p=0.03) in Japanese men (n=509, age 30-100) [67]. The biological role of the complex is not known; it may prevent C1q from activating the complement cascade, but as a side effect may prevent adiponectin from performing other beneficial functions.
Based on a twin study, having higher levels of adipose tissue leads to decreased levels of adiponectin and an upregulation of early complement components, including C1q, in adipose tissue [68]. The increase in C1q in the context of obesity may be a compensatory response to increased inflammation and play a role in the clearance of lipids and apoptotic debris. Anti-C1q therapy is expected to target free C1q, but spare complexed C1q. It is not known how this would influence CVD.
In CAD patients taking antiplatelet medication (clopidogrel therapy), serum C1q levels were associated with ADP-induced platelet aggregation and was a predictor for high residual platelet reactivity (OR: 2.362, 95% CI 1.631 to 3.421; 4^{th} quartile vs. 1^{st} quartile C1q levels) [69].

Atherosclerosis: In the vasculature, C1q plays a role in the opsonization of modified lipids, and has a similar profile to the opsonin Mfge8 (see Mfge8 report) in terms of preventing plaque formation/promoting clearance while also promoting arterial wall thickening. The beneficial effect on lipoprotein clearance involves the ability of C1q to modify macrophage polarization [70]. C1q opsonizes oxidized or acetylated LDL, and uptake of these C1q studded lipid particles alters the transcriptional profile of the macrophages to a state that is anti-inflammatory, anti-apoptotic and promotes efferocytic activity [71; 72]. C1q can also bind to AGEs and facilitate their removal [73]. This suggests that C1q may be protective against early atherosclerosis, which is further supported by evidence of increased lesion size and apoptotic cell accumulation in the vessels of C1q deficient mice.
However, similar to the CNS, these protective effects are likely to be dependent on the local environment, with respect to which prospective binding partners are present, and whether other downstream components of the complement pathway are upregulated in a given tissue. In a high complement environment, high C1q may instead promote inflammation and plaque buildup. C1q can also adversely affect arterial remodeling in a complement-independent manner that involves Wnt

activation [74], similar to what is seen in skeletal muscle. This process is stimulated by angiotensin II, suggesting that angiotensin inhibitors may exert some of their beneficial effects by reducing C1q [60; 75]. Therefore, anti-C1q therapy could potentially promote atherosclerosis in young healthy people, but is likely to be beneficial in older individuals with ongoing vascular inflammation and plaque buildup. Consistent with these preclinical studies, elevated C1q was found to be associated with measures of arterial stiffness with age in a cross-sectional study of 127 healthy volunteers [76]. Increases in serum levels of C1q, TNF-α, and IL-6, as well as carotid-femoral pulse wave velocity, a measure of arterial stiffness, were seen in participants over age 40. C1q was found to be an independent predictor of pulse wave velocity after adjustment for confounders, and similar trajectories as both parameters tended to show gradual increases starting around age 30. The increases in C1q were also correlated with increases in systolic and diastolic blood pressure in this cohort.

Stroke: Due to disruption to the BBB, circulating markers of neuronal injury can be transferred from the CNS into the systemic circulation. Observational studies suggest that C1q levels may rise in the peripheral blood following acute neural injury, and that the degree of elevation may be related to the extent of damage [11]. A prospective observational study found that serum levels of C1q were elevated in patients following acute ischemic stroke (n=647) relative to controls (n=647) [77]. In stroke patients, the C1q levels were associated with the infarct volume and degree of neurological deficit, based on the National Institute of Health Stroke Scale (NIHSS). A separate prospective observational study similarly found that plasma C1q levels were increased in patients following acute primary intracerebral hemorrhage (n=101) relative to controls (n=101) (median: 225.04 mg/L, 25^{th}-75^{th} percentiles: 156.10 to 280.15 mg/L versus 88.18 mg/L, 70.12 to 117.69 mg/L) [78]. Once again, patients with higher C1q levels showed worse prognosis, such that levels higher than 270.11 mg/L were associated with worse prognosis at three months (OR: 4.821, 95% CI 1.211 to 19.200). The C1q level was also associated with the Glasgow coma score and hematoma volume, and showed similar prognostic capacity as these measures. In this context, C1q would be expected to facilitate both the clearance of cellular debris as well as the induction of potentially damage-exacerbating inflammatory complement activation, thus it is unclear whether there would be net benefit or harm from inhibiting C1q. It may depend on the stage, such that early on, C1q may foster clearance, while continued complement activation may drive inflammatory damage.

Glaucoma: C1Q IS ASSOCIATED WITH EARLY DISEASE PATHOLOGY
C1q mRNA and protein have been found to be elevated in the retina from glaucoma patients, as well as in monkey and rodent glaucoma models [79]. The upregulation of C1q is indicative of the loss of

dendrites and synapses, and precedes retinal ganglion cell loss, suggesting that it is an early stage in the degenerative process. Mice lacking C1q were protected from synapse loss in the DBA/2J mouse model of glaucoma, and a C1 esterase inhibitor was neuroprotective in a rat model [80].
ANX-007, an ophthalmic formulation of an anti-C1q Fab fragment has been tested in a Phase 1a open-label trial (NCT03488550) in nine patients with primary open-angle glaucoma (mean Humphrey visual field deviation between −3 and −18 decibels) via a single intravitreal injection of 1.0 mg, 2.5 mg, or 5.0 mg, and in a double-masked, randomized, sham-controlled Phase 1b trial (NCT04188015) in 17 patients with primary open-angle glaucoma (mean Humphrey visual field deviation between −3 and −24 decibels) using two doses of 2.5 mg or 5 mg ANX007 four weeks apart [81]. Best-corrected visual acuity logarithm of the minimum angle of resolution transformed decreased in the study eye, which represents improved visual acuity, in the sham and 2.5 mg dose groups, but increased (worsened) in the 5 mg ANX007 group, though the sample sizes were too small to draw meaningful conclusions. Levels of unbound C1q were undetectable in both ANX007 dose groups after 29 days, indicating strong target engagement.

Age-related macular degeneration: C1Q IS ASSIOCIATED WITH DISEASE PATHOLOGY
Geographic atrophy is characterized by progressive degeneration of the retinal cells in the macula secondary to age-related macular degeneration (AMD) [82]. During this process there is a buildup of lipid-rich drusen, which stems from the buildup of uncleared cellular waste material, such as damaged membranes. The drusen are rich in C1q-activating substrates, and C1q has been detected in close proximity to drusen deposits in retinal tissue from patients with AMD [83]. The complement cascade has been implicated in the pathogenesis of AMD. Variants in complement-related proteins account for approximately half of the characterized genetic risk factors for AMD [82]. Furthermore, variants that increase complement activity are associated with increased risk, whereas variants that reduce complement activity are associated with decreased risk. Clinical trials testing components of the alternative complement pathway have been unsuccessful, while trials targeting downstream effector components common to multiple complement pathways have shown evidence for efficacy, but are hampered by their therapeutic safety profiles. These trials suggest that complement-associated pathology in AMD may be mediated by the classical complement pathway, and that targeting upstream components, such as C1q, may offer a better therapeutic profile. Preclinical studies have shown that intravitreal delivery of the anti-C1q antibody, ANX-M1, mitigated photoreceptor cell death when administered seven days following photo-oxidative damage, but had no protective effect when it was administered prophylactically or systemically [83].

ANX007 is currently being tested in a randomized, double-masked, sham-controlled Phase 2 clinical in patients with geographic atrophy secondary to AMD (n=270) (NCT04656561).

Cancer: HIGH C1Q IS ASSOCIATED WITH WORSE PROGNOSIS

C1q expression is associated with tumor growth and aggressiveness in some tumor types. Elevated C1q levels have been associated with poor survival in a variety of cancers [84]. In breast cancer patients, a high level of C1q was found to be associated with poor prognosis [85]. **C1q may play a role in modifying the tumor microenvironment in a manner that promotes tumor growth** and spread in a complement-independent manner. In tumor-associated tissue (melanoma, colon lung, breast, and pancreatic adenocarcinoma n=6 for each type), C1q was found to be primarily expressed in vascular endothelial and fibroblast cells and in infiltrating monocytes, and was associated with tumor invasion [86]. The ability to promote the proliferation and migration of endothelial cells promotes tumor angiogenesis and metastasis. C1q binds to a variety of ligands in the extracellular matrix, including hyaluronic acid [87]. In mesothelioma cells, C1q enhanced cell adhesion, migration, and proliferation through activation of ERK1/2, JNK, and p38 signaling pathways. C1q was found to be a marker of a subpopulation of suppressive tumor-associated macrophages (TAMs), and the presence of these macrophages correlates with levels of tolerogenic exhausted T cells in the tumors. The opsonization of C1q-tagged dying cancer cell debris may mediate the priming of tumor antigens to regulatory T cells to facilitate tolerance rather than attack toward the tumor cells [84].

However, C1q has been shown to either promote or inhibit cancer growth in different preclinical cancer models, suggesting that tumor type and microenvironment conditions may be critical for determining whether a given patient may benefit from anti-C1q therapy [88].

Liver disease: C1Q IS ELEVATED IN INFLAMMATORY LIVER DISEASE

In a study including 91 patients with chronic liver disease, serum C1q levels were higher in patients with liver cirrhosis relative to those with hepatitis, and C1q levels over 11 mg/dL were associated with a shorter duration of survival [89]. Serum C1q levels were inversely associated with markers of liver function, such as albumin, choline esterase, and platelet counts, and positively associated with markers of liver fibrosis, such as hyaluronic acid, PIIINP, and TIMP-1. C1q can promote the production of connective tissue growth factor (CTGF) via the induction of alternative Wnt signaling, which contributes to liver fibrosis [89]. Aging-associated increases in systemic inflammation can impact stem cell niches, and contribute to a reduction in productive tissue regeneration. Chronic inflammation, such as in the context of infection, can lead to a reduction in the renewal capacity of mature hepatocytes, leading to a compensatory activation of progenitor cells around the portal vein [90]. The expansion of this

population can be a driver of tumor formation. In mice, the C1q-mediated induction of Wnt/β-catenin signaling promoted the proliferation of this progenitor cell population [90]. This is consistent with the pattern of C1q expression in liver tissue from patients with hepatocellular carcinoma, in which C1q was enriched in the periportal area in close proximity to this progenitor population. In mice with liver inflammation, C1q inhibitors mitigated the proliferation of these progenitors and limited tumor formation.

C1q can interact with the lipoprotein ApoE. In the periphery, ApoE is abundantly expressed in the liver, primarily by hepatocytes, whereas C1q is expressed by Kupffer cells, a phagocytic resident macrophage population in the liver [91]. This interaction has been detected in multiple tissues, including the brain, vasculature, and synovial fluid, and the impact of this interaction can vary in a context-dependent manner, particularly with respect to how ApoE is modified [36]. Depending on where the interaction occurs, ApoE can either facilitate the recruitment of complement inhibitors, or can promote complement activation. In the context of inflammatory liver disease, the presence of ApoE-C1q complexes appears to serve as an indication of complement pathway overactivation [91]. ApoE binds with high affinity to activated C1q. Lipids, particularly oxidized lipids can activate C1q, and ApoE-C1q complexes were found in high density in areas with high levels of extracellular lipid droplets in the livers of patients with non-alcoholic fatty liver disease (NAFLD) [91]. In patients with viral hepatitis, ApoE-C1q complexes, and complement activation were abundant near the hepatic portal vein system, which has high levels of immune cell infiltration. These studies suggest that elevated C1q may be a driver of inflammatory damage and carcinogenic transformation in patients with chronic inflammatory liver diseases, such that blocking C1q downstream activity may be hepatoprotective.

Safety: Systemic C1q antibodies may increase the risk for autoimmunity and infection in predisposed individuals. They have shown a favorable short term safety profile. Local infusion/injection site reactions have been the most common adverse event in clinical trials.

Types of evidence:

- 5 clinical trials for ANX005
- 2 Phase 1 clinical trials for ANX007
- 1 Phase 1 clinical trial for ANX009
- 1 Phase 1 clinical trial for ANX1502
- 1 Preclinical toxicology study for ANX005
- 1 Preclinical toxicology study for ANX007

- Numerous studies on role of complement cascade in immunity
- Several observational studies on association of C1q with lupus

ANX005: Preclinical studies suggest that the recombinant humanized IgG4 anti-C1q monoclonal antibody (mAb), ANX005, does not induce overt toxicity in rats or monkeys at doses up to 200 mg/kg [3]. There were no significant sex differences in the pharmacokinetics, although the half-life was slightly different. In monkeys the Cmax in the serum was two hours (peak levels 2000 ug/ml at 100 mg/kg). Low doses (15 mg/kg) were cleared within five days, while the high doses (100 mg/kg) took 20 days to clear. CSF levels were 0.04 to 0.11% of levels in the serum for high doses, and undetectable at low doses (≤50 mg/kg). At doses of 100-200 mg/kg, the levels of ANX005 antibody were found to be sufficient to fully occupy C1q in the CSF. ANX005 CSF levels were in the range of 100-200 ng/ml, while in humans, C1q levels are normally in the 20-200 ng/ml range, suggesting that doses of 50-200 mg/kg should be sufficient to neutralize C1q in humans. However, at these levels, ANX005 also reduced free C1q in the serum to undetectable levels, suggesting that ANX005 would exert significant effects on complement activity in the periphery at levels necessary to have therapeutic benefit in the brain. Additionally, anti-drug antibodies were detected in the animals treated at high doses, and these antibodies negatively impacted the drug response. The modification of the anti-C1q mAb to include a BBB molecular Trojan horse could potentially increase BBB penetration and allow for a lower systemic therapeutic dose [92], but there is currently no evidence that efforts are being made to enhance the BBB penetration of ANX005. Instead, Annexon is developing a next-generation monoclonal antibody targeting C1q, ANX105, to have properties that allow for more favorable dosing and CNS penetrance (Corporate Presentation).

ANX005 was tested in a Phase 1 RCT (NCT03010046) in healthy volunteers (n=27) as a monotherapy or in combination with IVIg in a single ascending dose via intravenous infusion. According to Clinicaltrials.gov, ANX005 was well-tolerated at the doses used in the study, but the trial was terminated by the sponsor (Annexon), in order to initiate a trial in the relevant patient population (Guillain-Barre Syndrome). A single dose of ANX005 (75 mg/kg i.v.) was then tested in an open-label Phase 1b trial in patients with Guillain-Barre Syndrome (GBS-disability scale of 3–5) (n=14) in combination with IVIg (2 g/kg over 5 days) (NCT04035135). The combined treatment resulted in full C1q target engagement for one to three weeks [93]. In a single ascending dose study, ANX005 infusions up to 75 mg/kg were well-tolerated and there were no treatment-related serious adverse events [94]. The most common treatment-emergent adverse events were infusion-related reactions in the form of low-grade rashes. In a Phase 2 trial in patients with early manifest HD, ANX005 was generally well-tolerated, with transient first dose infusion-related reactions as the most common adverse event (Press release). There were

three discontinuations for possible drug-related reasons, including an event of systemic lupus erythematosus, an event of idiopathic noninfectious pneumonitis, and a case of asymptomatic hemolytic anemia, which all resolved or improved upon drug cessation. All three cases involved patients with elevated antinuclear antibody titers at baseline. There were no cases of serious infections. An analysis of safety data from 116 participants across studies testing ANX005 found that the most common treatment emergent adverse events were infusion-related reactions (32.8%), which was largely restricted to the first dosing event and the next most common event was headache (31.9%) (Corporate Presentation). Elevations in creatine kinase were seen in both ANX005 and placebo groups in patients with Guillain-Barre Syndrome, which is consistent with Guillain-Barre Syndrome.

ANX007: Since ANX007 is injected directly into the eye (via intravitreal injection), it is expected to have less systemic effects and a more favorable therapeutic profile. In a Phase 1a single ascending dose study (n=9) (NCT03488550), ANX007 was tested at doses up to 5 mg, and found to be well-tolerated. The most common ocular treatment-emergent adverse events were ocular irritation, conjunctival hemorrhage, and conjunctival hyperemia, and were not dose-related. There was an event of sinusitis that was not considered to be drug related [81]. In a Phase 1b multiple ascending dose study (n=17) (NCT04188015), treatment-emergent ocular adverse events included conjunctival hemorrhage, conjunctival hyperemia, eye irritation/pain, foreign body sensation in the eyes, ocular hyperemia, and blurry vision, which were also not dose dependent. A case of bronchitis was not related to the study drug. There were no abnormal findings on laboratory tests in either study. A preclinical study conducted in cynomolgus monkeys testing single bilateral intravitreal doses up to 5 mg or repeated bilateral doses up to 5 mg found that ANX007 specifically inhibited the classical complement pathway, and not the alternative complement or lectin pathways at clinically relevant doses [4]. Vitreous levels of the free drug were detectable up to four weeks following an injection, suggesting that monthly dosing would be appropriate. Levels of free drug were correlated with the degree of C1q target engagement. The drug reached retinal tissues via diffusion, such that areas farthest from the vitreous, such as the optic nerve, had the lowest drug levels. In this study, no adverse events related to ANX007 were noted.

ANX1502: Preliminary results from an ongoing Phase 1 trial testing ANX1502, an oral small molecule inhibitor of C1q, in healthy volunteers (n=84) (NCT05521269) found that the drug was well-tolerated at all tested single doses, and the maximum tolerated dose has not yet been reached. All treatment-emergent adverse events were mild and moderate, and there were no abnormal clinical or laboratory findings (Corporate Presentation).

ANX009: A Phase 1 single and multiple ascending dose trial tested ANX009, an antigen binding fragment (Fab) of a humanized antibody against C1q that has been formulated for subcutaneous administration, in healthy volunteers (n=48) (NCT04535752) [5]. A dose-response relationship was observed with respect to C1q inhibition. ANX009 was generally well-tolerated, and treatment emergent adverse events primarily consisted of mild, transient, local injection site reactions. There were no serious adverse events or dose-limiting toxicities.

Risk for autoimmunity: C1q deficiency is associated with increased risk for the autoimmune disease systemic lupus erythematosus [95], and the presence of serum anti-C1q antibodies can be part of the diagnosis for lupus, particularly in patients with renal pathology [96]. Apoptotic cell debris is considered a source of autoantigens for lupus. C1q can facilitate the phagocytic uptake of apoptotic cells in a complement-independent manner, thus C1q deficiency can hamper clearance [88; 97]. C1q is also involved in preventing autoimmunity by polarizing phagocytic cells to regulate effector immune cell activation. This suggests that a severe depletion of C1q could potentially lead to an increased risk for lupus, but further safety testing is needed to determine if the level of C1q reduction associated with therapeutic doses of anti-C1q antibody therapy would significantly increase the risk for an autoimmune disease.
While it may increase risk, anti-C1q therapy is expected to mitigate rather than exacerbate disease severity in individuals with pre-existing autoimmune diseases. C1q facilitates the clearance of antibody-antigen immune complexes. In autoimmune diseases, deposition of autoantibody-antigen complexes on healthy cells can drive complement-mediated cytotoxicity, leading to extensive tissue damage. In preclinical studies, anti-C1q treatment was able to reduce complement-mediated damage in rodent models of Guillain-Barre Syndrome and Neuromyelitis Optica [98; 99]. ANX009 is being developed for autoimmune conditions, including lupus.

Risk for infection: The complement pathway is part of the innate immune system and is important for regulating components of both the innate and adaptive immune systems. C1q has a recognition motif for the Fc part of IgM and IgG antibodies and initiates the antibody-mediated killing of pathogens through activation of the downstream complement cascade [88]. Loss of this system is associated with an increased risk for infection, particularly by bacteria. C1q is the initiating component of the classical complement system, but is not involved in the lectin or alternative pathways, which also converge on downstream complement components. Therefore, targeting C1q is expected to specifically affect the classical complement system, while sparing the others, and thus have a lower risk for infection than therapeutic agents targeted to downstream components. The C5 inhibitor, eculizumab, contains boxed

warnings for fungal, meningococcal, and streptococcal infections, and is contraindicated in people unvaccinated against Neisseria meningitis (PDR.net).

Drug interactions: The extent of drug interactions with anti-C1q is not yet known, but is expected to overlap with that of other complement component inhibitors, such as the C5 inhibitor eculizumab.

Sources and dosing:

Anti-C1q antibodies are being developed for therapeutic use for neurodegenerative and autoimmune diseases by Annexon Biosciences and are currently being tested in clinical trials. Therapeutically safe and effective doses have not yet been established for any indication. ANX005 is a recombinant humanized IgG4 anti-C1q monoclonal antibody used in an intravenous formulation and ANX105 is a next-generation monoclonal antibody toward C1q formulated for intravenous administration. ANX007 is an antigen binding (Fab) fragment that targets C1q used in an ophthalmic formulation administered via intravitreal injection. ANX009 is a Fab fragment targeting C1q designed for subcutaneous administration, and ANX1502 is an orally bioavailable small molecule inhibitor of C1q. They are currently only available for human use in clinical trials.

Preclinical studies have shown that angiotensin II inhibitors can reduce levels of C1q, and its deleterious effects on vascular remodeling and skeletal muscle repair [60; 75]. Exercise has also been shown to reverse the age-related increases in C1q [37; 55; 59].

Research underway:

ANX005 is currently being tested in a Phase 2 trial in patients with ALS (NCT04569435) which is expected to be completed in late 2023-early 2024, and in a Phase 3 trial in patients with Guillain-Barré Syndrome (NCT04701164) that is expected to be completed in mid-2024. A Phase 2/3 trial for ANX005 in Huntington's disease is also planned (Press release).

ANX007 is currently being tested in a Phase 2 trial in patients with geographic atrophy secondary to age-related macular degeneration, which is expected to be completed in mid-to-late 2023 (NCT04656561).

ANX009 is currently being tested in a Phase 1 trial in patients with lupus nephritis, which is expected to be completed in mid-to-late 2023 (NCT05780515).

ANX1502 is currently being tested in a Phase 1 single and multiple ascending dose study in healthy volunteers, which is expected to be completed in mid-2023 (NCT05521269).

ANX105 is currently being tested in a Phase 1 single ascending dose study in healthy volunteers, which is expected to be completed in mid-2023 (NCT05288881).

Search terms:

Pubmed, Google: C1q, Anti-C1q, ANX005, ANX007, ANX009, ANX1502 +

- Alzheimer's disease, neurodegeneneration, aging, cardiovascular, diabetes, inflammation, autoimmunity, cancer, glaucoma, safety, pharmacokinetics, clinical trials

Websites visited for Anti-C1q:

- Clinicaltrials.gov (ANX005, ANX007, ANX009, ANX1502, ANX105)
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