 doi:10.20944/preprints202109.0437.v1

How Senescent Glia Drive And Underlie Alzheimer's Disease: A Predictive Model

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 doi:10.20944/preprints202109.0437.v1

Abstract

Alzheimer's disease (AD) predominantly occurs as a late-onset form (LOAD), involving neurodegeneration and cognitive decline with progressive memory loss. Over time, risk factors and aging promote accumulation of well-known AD hallmarks in oxidative stress, amyloid-beta and tau protein pathology, as well as inflammation. Homeostatic glial functions regulate and suppress these AD hallmarks; however, other glial states involve increased pro-inflammatory cytokine release and further hallmark accumulation. Different stresses can additionally induce cellular senescence, or an irreversible differentiation process resulting in decreased supportive functions and increased, pro-inflammatory cytokine release. While these pathophysiological underpinnings all contribute to LOAD, they require temporal and mechanistic integration. This Perspective proposes that traditional AD hallmarks induce glial senescence in LOAD, where sufficient senescent glia exacerbate ongoing AD pathology and primarily drive LOAD into clinical, cognitive decline. We first explore age-related increases in pro-inflammatory glial activity, and then discuss emerging evidence linking oxidative stress, neurons containing tau pathology, and amyloid-beta to microglia, oligodendrocyte progenitor, and astrocyte senescence. Our evidence-based model mainly predicts that senescent astrocytes and oligodendrocyte progenitors together pressure microglia to phagocytose neurons containing tau pathology, where resulting senescent microglia create neuritic plaques and induce paracrine senescence transitioning into and progressing clinical, dementia presentation. This predictive model accounts for why medications used to treat LOAD fail, as previous treatments have not reduced senescent glial burden. It is also coherent with the predominant hypotheses surrounding LOAD involving the amyloid cascade, tau, glia and inflammation, creates testable hypotheses about LOAD, and increases rationale in testing senolytics as targeted treatments for LOAD arrest and reversal.

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Introduction

Alzheimer's disease (AD) involves cognitive decline, most prominently manifested as progressive memory loss. Risk factors linked to AD include vascular pathology, declining metabolism, and most dramatically, aging[1-4]. These risk factors contribute especially towards the highly-prevalent (>90% of AD cases) sporadic or late-onset AD (LOAD), and to a lesser extent to the early-onset or inherited, familial AD (EOAD)[3]. In LOAD, synaptic loss is pathologically correlated with cognitive decline[1,2]; however, little mechanistic insight is available to drive the development of effective therapeutics. Widely accepted explanations for neuronal loss and tracking LOAD progression have been proposed in the tau hypothesis and amyloid cascade[2] (Box 1).

Box 1 Dominant LOAD theories

In the tau hypothesis, upstream enzymes phosphorylate multiple residues in tau protein to create hyperphosphorylated tau (hp-tau)[1,3]. Hp-tau starts as monomers, but undergoes oligomerization and forms filaments, neuropil threads, and neurofibrillary tangles (NFTs). Neuritic plaques are compact amyloid plaques that also contain both hp-tau and dystrophic neuronal elements[1,3,5]. Hp-tau aggregation is equivalently classified under "neurofibrillary pathology" or degeneration, where neurofibrillary degeneration has been clinically documented into a set of six Braak stages[5]. In stages I and II, initial neurofibrillary pathology spreads from the transentorhinal region into the entorhinal cortex and hippocampus. Stages III and IV involve increased lesions and pathologies in these areas, and novel spread into the temporal lobe and insular cortex. Stages V and VI affect the remaining superior temporal gyrus and neocortex. Finally, Braak staging of hp-tau pathology has been well shown to correlate with AD progression[1,5,6].

In the amyloid cascade, accumulation of amyloid-beta (Aβ) protein, especially Aβ oligomers, act through multiple pathways to cause neurodegeneration and synaptic loss[2,3]. Misfolded Aβ monomers undergo a slow 'seeding', nucleating phase into damaging Aβ oligomers in AD, which then further aggregate into insoluble Aβ fibrils and diffuse amyloid plaques without hp-tau aggregates[3]. Misfolded hp-tau and Aβ can also destabilize their respective native conformations and spread equivalently as prions[3]. Both protein pathologies also add to each other (Fig. 1). However, diffuse amyloid plaques and insoluble Aβ fibrils do not correlate well with AD progression[1,5].

More theories of LOAD also exist, primarily through impaired metabolism by a combination of increased insulin resistance, hypertension, obesity, and/or insufficient sleep[3,4]. Vascular dementia, consisting of progressive blood supply blockades, cerebral amyloid angiopathy and Aβ deposition into cerebral blood vessels, likely leads to and/or correlates with LOAD incidence[4]. Finally, all these hypotheses involve a neuroimmune component; inflammation caused by pro-inflammatory cytokine secretion increases during LOAD, as a result of accumulating protein aggregation and declining cell metabolism[2-4].

Although exploring each classical AD paradigm has significantly improved progress towards understanding AD, no clinical trial targeting these pathologies has successfully halted AD[3,4]. A recent shift has geared towards understanding AD by focusing on next generation sequencing, aging-related pathology, and glia, or the non-neuronal brain cells. Particularly, many new genetic risk factors including *TREM2, CR1,* and *APOE* have exclusive roles in glia[3]. Some

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genes mediate increased phagocytic function in these glia, whereas other genes allot glia towards adopting pro-inflammatory states leading to chronic damage and cell death. Furthermore, glia also decrease neuronal support by undergoing senescence[7,8]. Cellular senescence, an irreversible cell arrest caused by environmental stresses such as DNA damage and oxidative stress, enables cells to gain apoptotic resistance. Although not universal, senescent cell markers include increased $p16^{INK4A}$, senescence-associated beta-galactosidase (SA-ß-gal), lipofuscin accumulation and transient p21 upregulation[7,8]. Senescent cells also adopt a senescence-associated-secretory-phenotype (SASP), which generates a pro-inflammatory profile initiated by cGAS-cCAMP-STING pathway signaling and subsequent cytokine, matrix metalloproteases (MMPs), and other protein secretion that can vary across different cell types[9,10]. Senescence moreover impairs most cellular functions and roles outside of wound healing, and reliably correlates with aging and accelerated mortality[10,11].

Altogether, LOAD is posited here as an aging disease of glial senescence. LOAD pathology is first explained through an aging lens, followed by evidence of glial dysfunction and decline in LOAD. Novel mechanisms involving AD neurodegeneration and aged glia are then discussed in an evidence-based framework that integrates Aβ aggregation, hp-tau, and synaptic-loss pathology.

Part I: Late-onset AD depends on aging and glial dysfunction

Aging and oxidative stress are understated risk factors in dominant LOAD paradigms

In all cells, oxygen intake and reactions generate free radicals that accumulate as reactive oxygen and nitrogen species (RONS), such as nitric oxide and superoxide[11]. When RONS accumulation overwhelms antioxidant defenses, RONS irreversibly alter and damage nucleotides, lipids, and proteins in cells. This phenomenon, oxidative stress, progresses with age due to accumulated double-stranded DNA breaks, mitochondrial dysfunction, and declining efficiency of metabolic processes[7,8,10,11]. Oxidative stress propagates pathways that elevate Aβ production, tau hyperphosphorylation, and release of pro-inflammatory cytokines including IL-1 and TNF-α[2,9-13]. Over time, increased pro-inflammatory cytokine production becomes a chronic systemic "inflammation" that increases with aging, and in turn exacerbates cell damage and death. Many LOAD risk factors (such as vascular pathology, sleep impairment, and chronic stress) also result in increased RONS levels and chronic, systemic inflammation[2-4].

Oxidative stress damages all cell types, including neurons. Particularly, RONS inhibit phosphatidylserine (PS) lipid translocases, increase intracellular calcium, deplete ATP, and activate phosphatidylserine scramblases; this "flips" PS from the inner to outer cell membrane, where outer PS acts as an apoptotic or "eat-me" signal[14-17]. RONS also create "oxysterols" by oxidize membrane cholesterols. Oxysterols have been hypothesized to drive LOAD[18-21], where one such mechanism will be elaborated below in Part II. Overall, these pathological factors or hallmarks engage each other in a shared environment, encourage further release and accumulation of each hallmark, and exacerbate cellular damage in positive loops[2,3,7-9,11-14,19-21] (Fig. 1). Finally, patients must minimally be aged 65 to be potentially diagnosed with LOAD[1,3]. This is significant time towards accruing aging and increased exposure to oxidative stress, and reflects in increased systemic inflammation, oxysterols, and protein aggregation resulting from RONS interactions[3,18]. What then allows for healthy, aging cognition against elevation of traditional AD hallmarks in Aβ aggregation, neurofibrillary tau pathology, chronic systemic inflammation, and oxidative stress?

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CNS glia lose homeostatic capacity in aging

In the healthy CNS, multiple types of glia exist in multiple states and serve a dizzying array of functions, a subset of which can be labeled as "homeostatic". Homeostatic glial activities sequester and break down cellular products to preserve optimal nervous system function[22]; this includes products comprising traditional AD hallmarks (Fig. 1). This parenchymal maintenance is achieved by paracrine signaling and endocytosis. Homeostatic glial functions involve trophic factors and secretion of anti-inflammatory cytokines including IL-10 and TGF-ß, which minimize cell stress and pro-inflammatory glial reactions at least acutely[23-27]. These molecules mediate communication in bi-directional and overlapping loops of glia-neuron and glia-glia interactions, through which glia supporting one another facilitate neuronal activity and plasticity. Microglia, particularly, require OPC-mediated secretions via the TGF-β2-TGFBR2-CX3CR1 signaling axis to sustain homeostatic functions[24,25]. Astrocytes also release trophic factors and cytokines including IL-3 to reduce pro-inflammatory microglial states[28], and both glia endocytose neuronal, synaptic, and extracellular elements[14,20,29-35]. Some of these elements include the traditional AD hallmarks in Fig. 1[3,22]; for example, microglia phagocytose extracellular Aβ and tau through receptors such as TREM2 or IGF1R[3].

However, expression of receptors that facilitate clearing of Aβ and tau can also push homeostatic glia into "pro-inflammatory" states. Examples include the RAGE and LRP1 receptors, both of which activate the NLRP3 inflammasome[3,36-38]. While pro-inflammatory glia provide effective protection in acute illness and infection, their continuous and exaggerated reactions during aging contribute to cellular stress, death, and (indirectly) accumulation of traditional AD hallmarks[2,3,7]. The resulting environment gradually lowers homeostatic support for glia and may partially explain a "priming" effect, wherein aged glia expand and exaggerate pro-inflammatory responses towards smaller amounts of pro-inflammatory cytokines or Aβ[9,39,40]. In parallel, aging and exposure to RONS reduce homeostatic signaling, impairing the TGFßII-Smad pathway in aged microglia[33,34]. Aged glia additionally have increasingly impaired metabolism and endolysosomal systems[8,10,39,40], causing decline in phagocytotic capacity and clearing of Aβ as well as hp-tau accumulation. Thus, while homeostatic glial functions protect the brain for most of adult life, their ability to combat both increasing pro-inflammatory glial responses and traditional AD hallmarks wanes over aging and environmental risk factor exposure (Fig. 2).

Glial senescence is associated with LOAD progression

Multiple lines of evidence indicate that APP overexpression and subsequent Aβ hyper-accumulation is associated with exacerbated OPC senescence (Fig. 3). In APP/PS1 mouse models representing EOAD, OPCs surrounding amyloid plaques display senescence per dystrophic shrinkage in cell volume and immunopositive p21, $p16^{INK4A}$ and SA-ß-gal staining[41,42]. OPC numbers also decrease in APP/PS1 mice, and correlate with decreased myelin levels likely secondary to reduced oligodendrocyte differentiation and myelin generation[42,43]. In LOAD patients, global Aβ accumulation and increased numbers of p21+, senescent OPCs proportionally scale with progressive Braak staging[41]. This suggests that OPC senescence begins in early Braak stages and precedes clinical LOAD presentation, then continues to progress in parallel with LOAD (Fig. 2).

Astrocytes also undergo increased senescence in LOAD. Particularly, oxidative stress, Aβ, and tau have all been shown to induce human astrocyte senescence; senescent astrocytes exhibit a SASP profile, releasing pro-inflammatory cytokines and MMPs[44-46]. Senescent astrocytes also down-regulate potassium and glutamate transporters Kir4.1, EAAT1, and EAAT2. This results in increased extracellular glutamate, contributing to neuronal excitotoxicity and death[47], alongside

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NMDA-receptor signaling and pro-inflammatory behaviour in microglia[48]. Senescent astrocytes positive for $p16^{INK4}$ were additionally found to increase in human brains over the course of aging and be over-represented in frontal cortices from LOAD patients *versus* age-matched controls[45].

Increased OPC and astrocyte senescence likely exacerbate traditional AD hallmark burden (Fig. 3) via multiple effects: they can directly promote all LOAD hallmark accumulation (Fig. 1), weaken neuronal support, and increase pro-inflammatory states in other glia[7,8]. Astrocyte and OPC senescence also induce paracrine glial senescence, at least indirectly[7,49]. However, Aβ and/or oxidative stress can trigger OPC and astrocyte senescence without tau[41,42,44]. Thus, the senescence of these glia alone does not correspond well with neurofibrillary degeneration in Braak staging, and inadequately explains the preclinical to clinical LOAD transition. Another crucial role of OPC and astrocyte senescence during preclinical LOAD may be their impact on microglia: when senescent OPCs and astrocytes withdraw homeostatic support and exacerbate AD hallmark accumulation, they overwhelmingly exhaust and "prime" homeostatic microglia for increased pro-inflammatory reactions and phagocytosis of neurons[7,8,23-25,28,39]. Moreover, the preclinical to clinical LOAD transition putatively corresponds to increased microglial senescence and spread of neurofibrillary pathology, particularly in NFT and neuritic plaque formation (Fig. 2, Fig. 3).

Part II: Accumulating microglial senescence drives clinical progression of LOAD

Microglial senescence and priming in LOAD is evident in single cell transcriptomics

Transcriptomic studies of microglia in LOAD patients provide additional evidence for APOE priming and senescence. The largest LOAD microglial sample size for single cell RNA sequencing was examined by Olah *et al.*, where microglial clusters 7, 8, and 4 particularly upregulated *APOE*[50]. Clusters 4 and 8 also displayed increased ferritin in *FTH1* and *FTL*, and cluster 4 uniquely upregulated multiple *IRF* transcription factors. This indicates that microglial cluster 4 possesses an upregulated type I interferon (IFN) response to environmental pathology. Type I IFN signaling has been implicated in pro-inflammatory microglial responses to Aβ pathology across multiple mouse models[51-53], and is associated with plaque-associated microglia in LOAD patient samples[52]. In absence of an acute viral infection, it is notable that the cGAS-STING pathway in senescence also upregulates type I IFN responses[10,54]. Increased type I IFN responses, induced by senescence, may thus further exacerbate pro-inflammatory microglial reactions to Aβ pathology.

Further complementing these results, a microglial population observed in LOAD patients was separately validated both transcriptionally and by immunostaining per Nguyen *et al.*[55]. This population was found to increase both FTH and FTL expression, associate with neurofibrillary pathology, and particularly neuritic plaques, as well as display signs of dystrophy indicating senescence. Although dystrophy will be later explained when discussing senescent microglia in LOAD patients, this "dystrophic" microglial population was speculated to be the end result of microglial subsets responding to Aβ accumulation. These Aβ-responding microglia were also found to upregulate *APOE*[55]. As described later, this *APOE* upregulation likely confers a "primed" state before microglia become dystrophic (Fig. 3).

Microglial senescence is driven by phagoptosis of hp-tau-containing neurons

When microglia are primed to phagocytose neurons in LOAD, they likely enter a vicious pathological cycle: multiple lines of evidence suggest that phagocytosis of hp-tau renders microglia senescent. In a P301S tauopathy mouse model, which approximately models AD pathology through human tau overexpression and subsequent tau hyperphosphorylation, senescent

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microglia were identified with positive p16^{INK4A} and SA-ß-gal staining[56]. Both extracellular and intracellular hp-tau stress human neurons *in vitro*, induce outer PS exposure, and initiate premature neuron phagocytosis or "phagoptosis" by human microglia[57,58] (Box 2). When co-cultured with P301S neurons growing intracellular hp-tau filaments, human microglia phagoptosed the viable neurons and became senescent [70]. These senescent microglia exhibited increased NF-kß activation, MMP-3 release, and positive SA-ß-gal staining; additionally, they displayed hypo-phagocytic capacity, or poor phagoptosis of P301S neurons containing hp-tau aggregates, and aberrant release of insoluble hp-tau aggregates into the local environment[70]. In both humans and mice, plaque-associated microglia release soluble tau that can seed insoluble hp-tau aggregates[71-73]. Thus, tau seeding and release by senescent microglia can spread *in vivo* to induce a pathological cycle of neuronal phagoptosis and microglial senescence.

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Box 2: Microglial priming, ramified morphology, phagoptosis, and neurodegenerative synaptic loss in LOAD

While microglia in homeostatic states often comprise a ramified morphology, human ramified microglia do not always perform homeostatic functions. Contrarily, pro-inflammatory lipopolysaccharide has been shown to induce a complex, ramified morphology in human microglia[59]. Pro-inflammatory microglia states thus are not always equivalent to "activated" or hypertrophic, amoeboid-shaped microglia that cluster around neuritic plaques in LOAD patients[60-62]; more accurately, amoeboid or hypertrophic morphologies can indicate that human microglia have phagocytosed extracellular materials[61]. This hypertrophic appearance happens regardless of the microglia's inflammatory role, as later discussed.

Contrarily, in homeostatic function, microglia are shown to mainly utilize TREM2 in phagocytosing synapses and whole neurons for CNS development, plasticity, and maintenance[17,29]. While homeostatic microglia can phagocytose dead neurons to optimize the surrounding environment, they can also phagocytose neurons that are stressed but not yet apoptotic. This process is "phagoptosis"; a premature, non-apoptotic death through microglial phagocytosis[15,16]. Mechanistically, oxidative stress exposes PS at the neuronal outer cell membrane to act as a ligand. Simultaneously, microglia reacting to pro-inflammatory stimuli produce sialidase that removes or "desialylates" neuronal sialic acids[14-16]. As microglia enter pro-inflammatory states more frequently during aging[39], and sialic acids protect neurons from being phagocytosed[14-16], aging renders neurons more susceptible to phagoptosis. Thus, although microglia in pro-inflammatory states may not directly partake in phagoptosis while secreting pro-inflammatory cytokines, their actions "prime" microglia in homeostatic states to phagoptose neurons.

Once neuronal PS is sufficiently exposed, extracellular opsonin proteins coat outer PS and induce phagoptotic death by microglia. This process occurs via multiple ligand-receptor pairs that converge into the DAP12/TYROBP/KARAP pathway, stimulating downstream signaling and subsequent phagocytosis through TAM receptor tyrosine kinases in Mer and Axl[15-16]. Specific pathways leading into phagoptosis and DAP12 signaling include TREM2 binding directly either to PS, or extracellular APOE bound to PS[16,30-32,63]. Complement C1q is another opsonin that coats PS, allowing for subsequent C3b, iC3b complement binding and phagoptosis by CR1/CR3/CR4 complement receptor activation[64]. These complement receptors also conduct downstream DAP12 signaling in microglia[15,16]. A separate pathway alternatively exists where calreticulin opsonin bins to C1q, resulting in neurons that are phagoptosed by microglial LRP1 and induction of more microglial, pro-inflammatory responses[3,16].

Of relevance, some homeostatic microglial states in Aβ-overexpressing mice phagoptose neurons and correspondingly exhibit a neurodegeneration/disease-associated microglial phenotype (DAM or MGnD)[65-67]. The transcriptome includes an initial DAM1, involving *APOE* upregulation independent of phagoptosis activation, and a subsequent DAM2, which necessitates TREM2-dependent activation, and further upregulates *APOE* and *ITGAX* relating to CR4 subunit CD11c[66]. Further DAM2 subsets have also been found, with a pro-inflammatory subset up-regulating the Kv1.3 channel protein that mediates increased RONS and pro-inflammatory cytokine release[67]. Dark microglia represent another relevant state, which has been observed in mouse models of chronic stress, aging and Aβ pathology, as well as in patients diagnosed with schizophrenia[68,69]. Dark microglia display markers of oxidative stress and lipofuscin accumulation related to senescence[2,10]. They likely participate in phagoptosis; dark microglia have increased phagocytic inclusions and commonly enwrap processes around shrinking but viable neuronal elements[68].

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AD hallmark accumulation mediates microglial senescence in LOAD

In aging, increasing oxidative stress accelerates oxysterol production, recognized by microglial liver X receptor (LXR)[18-21,74]. Upon successful oxysterol and LXR binding, microglial *APOE* is upregulated[69]; this *APOE* upregulation may well correspond to DAM1 in mouse models, and speculatively shift microglia towards the transcriptomic cluster 7 discovered by Olah *et al.*[50,66]. While potential functions remain unelucidated, this may result in extra microglial APOE secretion; as oxysterol creation depletes unoxidized cholesterol, LXR-activated microglia may secrete APOE packaged with cholesterol to aid in myelin, neuronal, and synaptic phagocytosis[75]. Intriguingly, upregulated *APOE3/4* can act as a nuclear receptor in microglia (Fig. 3), further accelerating microglial aging, pro-inflammatory responses, Aβ aggregation, tau hyperphosphorylation, and phagoptosis in *APOE4* variants[76-80]. APOE secretion also leads to APOE-PS binding stressed, desialyated neurons[16]. Combined with traditional AD hallmark accumulation and progressive OPC and astrocyte senescence, microglial APOE would further pressure neurons to expose outer PS and accumulate tau hyperphosphorylation, while priming microglial states to phagotopse neurons (Fig. 3).

Particularly, microglial states expressing TREM2, performing homeostatic functions, and upregulating *APOE* likely bind PS-exposed neurons with hp-tau aggregates. This triggers neuronal and hp-tau phagoptosis through a combination of TREM2, complement receptors, and opsonins[16]. This TREM2-involved phagoptosis likely induces simultaneous senescence and a DAM2(-like) microglial transcriptome[65-67], which when correlating to human microglia data, may represent the transcriptomic cluster 8 identified by Olah *et al.*[50]. However, it is unknown whether the TREM2-dependent, DAM2 program itself initiates senescence, or if phagoptosing neurons with hp-tau simultaneously initiates separate DAM2 and senescence programs. Evidence regarding tau inducing senescence has been presented[70]; contrarily, phagoptosis of stressed neurons without hp-tau aggregates was not yet shown to induce microglial senescence. Therefore, it can only be currently assumed that a DAM2(-like) microglia state becomes senescent. Finally, extracellular Aβ aggregates and tau can both induce phagoptosis, although significantly increased PS exposure requires much more time and occurs relatively late in APP/PS1 mice at 9.5 months of age[81]. Thus, while Aβ aggregation can also lead to phagoptosis and potentially senescent microglia, microglial phagoptosis, DAM2 induction, and senescence caused by hp-tau uptake are much more specific to neurofibrillary pathology and LOAD progression[82].

The process of hp-tau-induced senescence is still unclear, but hp-tau may inhibit apoptosis acutely as a trade-off in initiating senescence[83]. NFT-containing neurons in both P301L mice and frontotemporal dementia patients exhibited increased senescence transcriptome scores, revealed by up-regulated senescence markers such as *CDK2NA*, *TNF*, and *IL-1β*[7,84]. This supports that stressed neurons may turn senescent, and expose PS prior to rendering microglia senescent. It also suggests a universal mechanism by which senescence can be triggered in neurons, astrocytes, and microglia by neurofibrillary, hp-tau pathology (Fig. 3).

Clarifying microglial senescence and dystrophy in LOAD patients

Although microglial senescence remains to be rigorously confirmed with multiple markers in clinical LOAD, patients were found to present dystrophic microglia indicating senescence. These dystrophic microglia display cytoplasmic fragmentation, swollen bead process extensions, and associate with and precede NFT pathology following Braak staging[6,62,85,86]. Dystrophic microglia increase TREM2, APOE, and ferritin expression, resulting in increased iron intake[86-88]; senescent

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cells selectively uptake and accumulate iron[89,90]. These microglia not only became more abundant with aging, but also significantly increased in number among human hippocampi from differing types of dementias *versus* age-matched controls[91].

Dystrophic microglia were found to associate with neuritic plaques and neurofibrillary tangles in LOAD patients[55], with morphological differences depending on their local environment. Dystrophic microglia displaying hypertrophic somas were observed away from neuritic plaques, whereas microglia showing dystrophy and an extremely hypertrophic, "amoeboid" appearance were found to associate with neuritic plaques[62]. This neuritic plaque association was particularly observed in the prefrontal cortex, leading to propose that hypertrophic and dystrophic microglia form around the preclinical to clinical LOAD transition[62]. This complements a previous study by Sheng, Mrak, and Griffin, showing that microglia in LOAD patients initially transform from a "primed" or ramified appearance to a hypertrophic or amoeboid, phagocytic morphology nearby neuritic plaques[61].

Finally, neuritic plaque formation and accumulation correlates well with Braak staging, and the overall LOAD clinical progression[1,60,62]. Experimentally, ablating microglia early and minimizing downstream microglial senescence also reduced neuritic plaque deposition in Aβ-overexpressing 5xFAD mice[92]. Altogether, this indicates that dystrophic microglia are not solely due to aging, but specifically involved in LOAD progression. Notably, dystrophic, hypertrophic microglia that associate with neuritic plaques can be named plaque-associated microglia. However, this term of "plaque-associated" microglia requires more precise definition. In LOAD patients, "plaque-associated" microglia can only be classified in association to neuritic plaques; human microglia in LOAD patients do not significantly associate with diffuse plaques[60].

Senescent microglia putatively transform morphology and drive neuritic plaque formation in LOAD

From here, we propose that primed microglia in LOAD patients initially comprise a ramified, or hyper-ramified morphology[59]. After phagoptosing neurons with hp-tau and becoming newly senescent, microglia may adopt a ramified or slightly hypertrophic morphology. Recalling that senescent microglia display hypophagocytic capacity for hp-tau neurons and have compromised endolysosomal systems[70], their endocytosed aggregates are likely not fully digested. The impaired capacity to further phagocytose hp-tau aggregates putatively leads to further NFT creation, as both microglial degeneration in humans and microglial senescence in mice precede NFT formation[6]. Here, senescent microglia are accordingly proposed to actively phagocytose, yet fail in degrading further Aβ and hp-tau aggregates[10,31,70].

We then predict that senescent microglia having performed phagoptosis transform into a dystrophic morphology, as both ramified and hypertrophic somas were found in dystrophic microglia with beaded processes[6,55]. If not too exhausted, senescent microglia likely attempt to digest Aβ and potential hp-tau aggregates. In terms of preference, Aβ oligomers were shown to bind with high affinity to TREM2[93], indicating that TREM2-Aβ interaction may become preferential in senescent microglia after phagoptopsis. APOE also binds to Aβ oligomers[74,80], presenting an alternative TREM2-APOE-Aβ endocytic pathway for senescent microglia. Overall, this would induce senescent microglia to become bloated with these aggregates, displaying an amoeboid or extreme hypertrophic morphology correlating to microglial "activation" seen in LOAD patients[55,60-62] (Fig. 3).

While non-fibrillar, Aβ secretion may be useful in forming protective diffuse plaques, senescent microglia are predicted to fail in degrading these endocytosed products; instead, they

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putatively secrete Aβ-hp-tau aggregates with dystrophic neurites from phagoptosed neurons[1,36,71-73]. This is specific to senescent microglia, and critically accelerates buildup and spread of both Aβ and hp-tau[36,72]; these Aβ-hp-tau aggregates with dystrophic neurites then are predicted to aggregate into neuritic plaques[60,61]. Particularly, these secreted hp-tau and Aβ seeded aggregates are likely being uptaken by nearby microglia, to attempt limiting damage in a localized region. Albeit, this would render nearby microglia senescent too, creating neurofibrillary pathology that further causes a mobile, transcriptomic response in nearby microglia[55]. Hp-tau spread, paracrine senescence, and further aggregate seeding through these attracted microglia thus are posited to create the "plaque-associated" microglial clustering seen in LOAD patients. Furthermore, this overwhelming concentration of Aβ and hp-tau pathology in neuritic plaques also draws in, creates, and causes nearby "clustering" of more senescent astrocytes and OPCs[41,44] (Fig. 2).

Senescent microglia are thus putatively accountable for neuritic plaque formation, critical Aβ and hp-tau pathology beyond threshold tolerance in localized brain regions corresponding to Braak staging and LOAD progression[1,5,60,61]. Continued microglial exhaustion and attempted aggregate digestion may then result in dystrophic morphologies associated with LOAD, with subsequent negligence to protect neurons leading to further NFT creation[5,6,55,85]. Furthermore, dystrophic and potentially hypertrophic senescent microglia further respond to overwhelming Aβ aggregation with an increased type I IFN response[51-54], leading to pro-inflammatory cytokine secretion and *Kv1.3* expression exacerbating local inflammation[94-96]. These exhausted, senescent microglia may also correspond to the transcriptomic cluster 4 per Olah *et al.*[50], featuring increased *FTH1* and *FTL* expression implicating iron accumulation and senescence[55,88-91], *APOE* upregulation, and *IRF* transcription factors expression corresponding to an increased type I IFN response[51-54]. In mouse models with Aβ pathology, this microglial stage may correspond to a pro-inflammatory, TREM2-dependent DAM2 state in response to Aβ pathology[67,97]. Notably, TREM2 in hypertrophic and/or dystrophic states may also be downregulated due to pro-inflammatory exposure[98].

In LOAD, microglial clustering around neuritic plaques and symptom presentation emerge together around Braak stages III-IV[1,5]. Thus, it is predicted that senescent microglia actively accelerate hp-tau aggregates and form neuritic plaques that tip preclinical LOAD into clinical progression. Furthermore, the exacerbated, resulting localized concentration and accumulation of Aβ and neurofibrillary pathology determines clinical LOAD progression, gradually accumulating more synaptic loss, pro-inflammatory glial responses, and overall glial and paracrine senescence (Fig. 2).

Discussion

While the framework has not yet been empirically tested, its yields further understanding regarding LOAD and proposes many testable components[99]. This framework is compatible with current field paradigms regarding the amyloid cascade, tau, and synaptic loss hypotheses, and likely numerous other theories that have helped provide better knowledge of LOAD. It can also be expanded upon, as this perspective has not covered endothelial cell senescence and blood-brain barrier leakage contribution[100], other LOAD risk factors, declining metabolism in aged glia, and much more.

As an additional clarification, microglia also proliferate in response to accumulated Aβ pathology[35]. Over the course of aging, this further accelerates homeostatic microglial transition towards senescence through telomere shortening[7,8,101]. This may independently create senescent microglia that exacerbate Aβ pathology and chronic local inflammation, indirectly accelerating the conversion of senescent microglia through phagoptosing Aβ-stressed neurons containing hp-

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tau; alternatively, these senescent microglia may also accelerate tau seeding and spreading. This framework may further apply to EOAD, with one main exception: if glial and particularly microglial senescence are responsible for clinical AD progression, mutations responsible for EOAD may involve quicker microglial senescence through exhaustion. Particularly, with the TREM2 R47H mutation, microglia may have relatively decreased metabolic capacity to clear out quicker Aβ and consequent hp-tau buildup in EOAD[102,103]. Conjecturally, mutations could make it harder for other immune cells to clear out senescent microglial accumulation over aging[7].

Dark microglia may also be found to be senescent, provided that dark microglia likely phagoptose neurons[68]. Additionally, senescent microglia were absent from the inferior parietal cortex of LOAD patients in Braak stages I – VI[41]. Particularly, plaque-associated microglia were immunonegative for p21, and thus concluded to be non-senescent. As this contradicts the proposed framework, an alternative explanation is given. Foremost, although p21 has been used a senescence marker, p21 was shown to be transiently expressed after senescence induction[104]; furthermore, senescent human microglia were not yet characterized for their long-term expression of p21. It is thus recommended to confirm or reject the current framework by assessing microglial senescence in LOAD patients using multiple methods, such as lipofuscin accumulation, iron and/or SA-ß-gal staining, and a more constitutively-upregulated p16[7,10,101,104]. Moreover, the particular morphological and molecular signatures of senescent glia in LOAD should be further confirmed.

More broadly, tauopathies including chronic traumatic encephalopathy may represent a favourable target regarding treating senescent microglia and administrating therapeutics. LOAD pathology is also especially co-morbid with other dementias such as vascular dementia and Parkinson's disease with dementia[1], so solutions that target senescent glia in LOAD will likely help managing or treating symptoms in these other diseases. If the current framework is correct, increasing glial senescence explains why previous therapies have not worked; these medicines have not targeted senescent glia, and thus have not slowed consequent AD hallmark accumulation. A specific solution towards treating LOAD is encouraged here through senolytics, or drugs that selectively kill senescent cells (Fig. 4). As detailed, successful senolytic administration will likely treat the accelerated, traditional AD hallmark burden caused by senescent glia; this should allow renewal and recovery from remaining brain parenchyma, and for subsequent plasticity to extend or sustain an improved quality of life. While senolytics can or should be paired with other AD treatments for clinical trials, several senolytic experiments have demonstrated usefulness in preventing cognitive impairment in tauopathy and APP/PS1 mouse models[41,56,84]. The senolytics dasatinib and quercetin have also successfully eliminated senescent cells in a clinical trial for patients with diabetic kidney disease, purporting hope for treating LOAD as an aging disease of glial senescence.

 doi:10.20944/preprints202109.0437.v1

Acknowledgements
We acknowledge that the University of Victoria exists on the territory of the Lekwungen peoples and that the Songhees, Esquimalt and WSÁNEÆ peoples have relationships to this land. V.L. was supported by graduate award scholarships from the Faculty of Graduate Studies, University of Victoria. M.E.T. is a Tier II Canada Research Chair in *Neurobiology of Aging and Cognition.*

Author contributions
V.L. led the article conceptualization, manuscript writing, and figure creation; V.L., L.M., and M.E.T. critically discussed the content and contributed to editing and revising the manuscript.

Competing interests
The authors declare no competing interests.

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(Figures: All are intended to be 2-column).

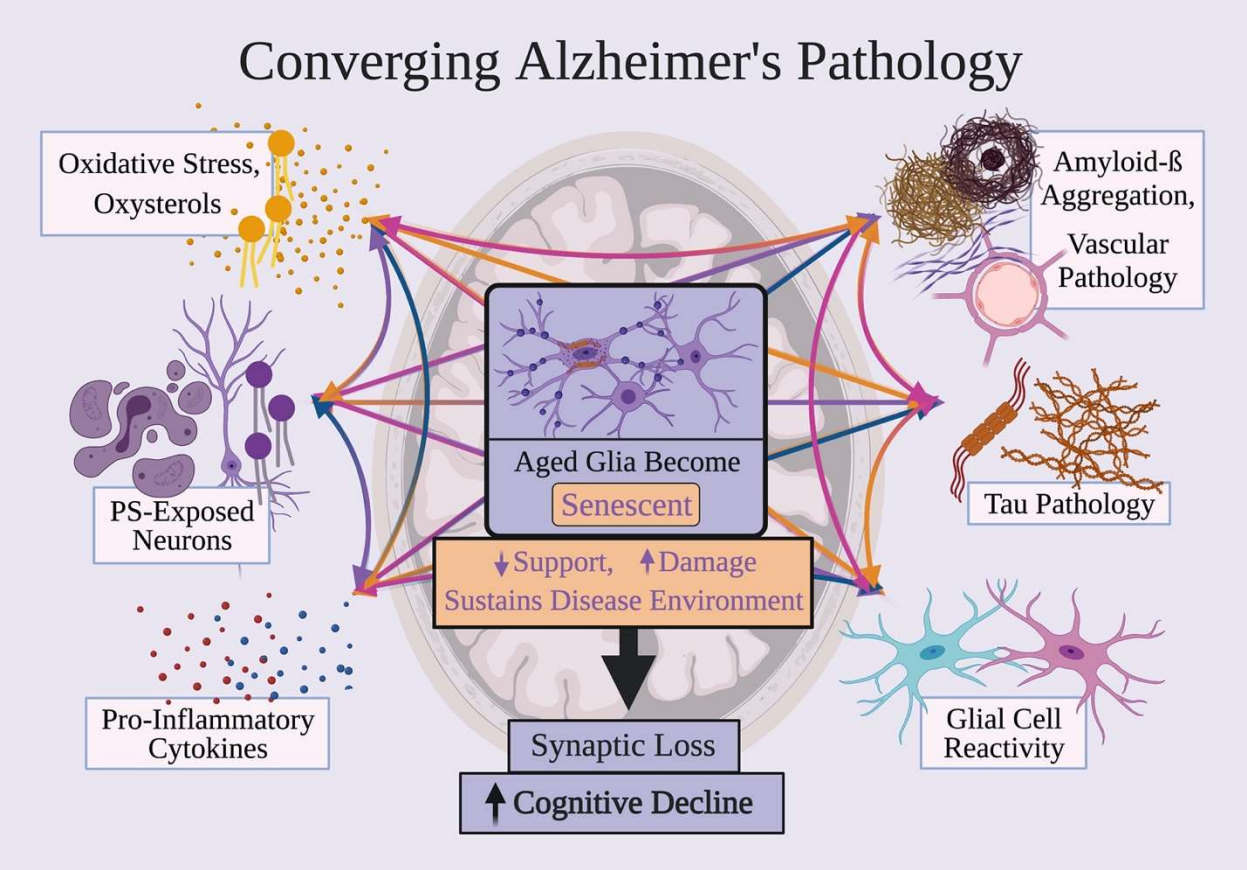

Fig. 1: Converging Alzheimer's Pathology. Risk factors for late-onset Alzheimer's disease (LOAD) end up adding to at least one of six main pathologies indicated in each corner, where each main pathology eventually adds to and increases the burden of other main LOAD pathology. These pathologies converge to ultimately drive improper neuronal support and death, resulting in synaptic loss clinically corresponding to cognitive decline. Centrally, overwhelming levels of these six LOAD pathologies also lead to threshold glial senescence. Glial senescence likely further enhances all six pathologies, promoting a disease environment favouring and irreversibly inducing synaptic loss and neuronal death.

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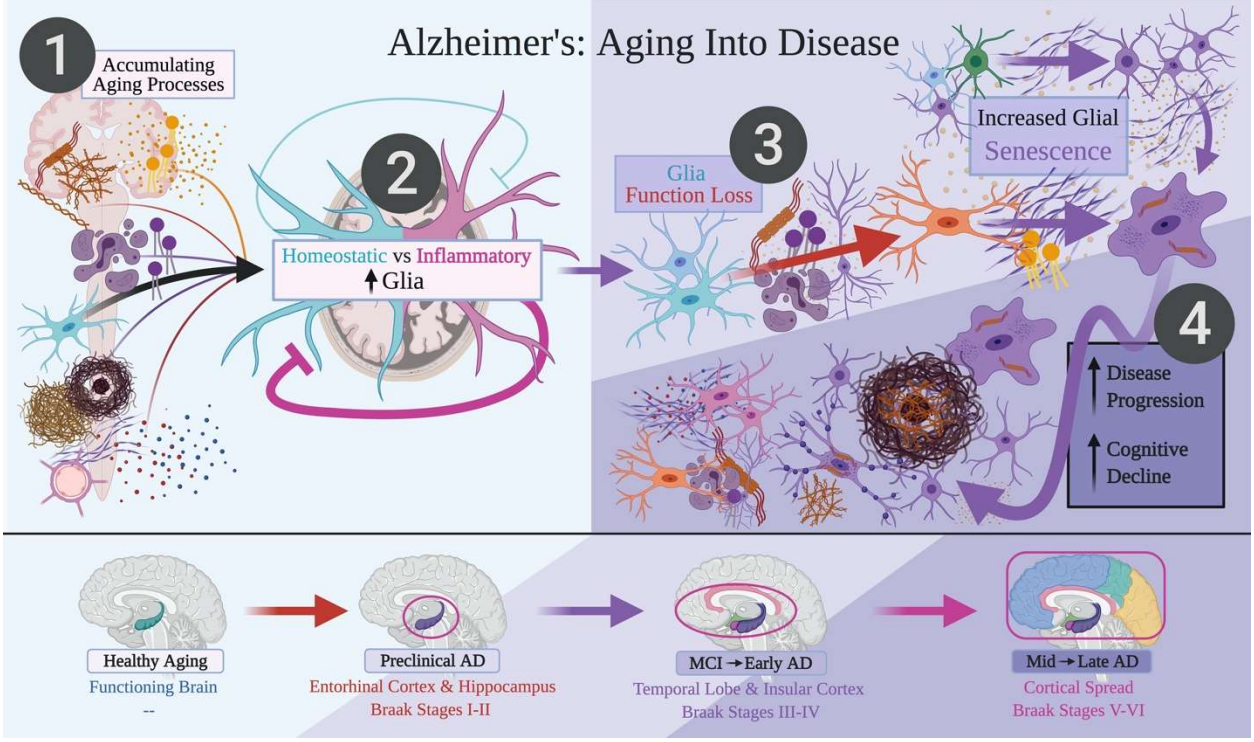

Fig. 2: Alzheimer's: Aging Into Disease. A LOAD framework is proposed from healthy aging to late-stage AD, presenting the testable claim that glial senescence accumulation directly corresponds to clinical LOAD progression. (1) Oxidative stress, amyloid-beta (Aβ), neurofibrillary pathology in hyper-phosphorylated tau (hp-tau), and chronic inflammation constantly accrue throughout healthy aging as by-products of central nervous system function and metabolism. (2) Main LOAD pathology levels are enhanced by inflammatory glial states, and are sufficiently cleared by glia performing homeostatic roles. However, homeostatic glial functions decline throughout aging and decrease proficiency in containing LOAD pathology. (3) In preclinical AD, increasing proportions of aged glia interact with LOAD pathology to become senescent. This includes oligodendrocyte progenitor cells and astrocytes. Microglia performing homeostatic roles become senescent after engulfing neurons containing hp-tau, and then become incompetent in breaking down further phagocytosed hp-tau and Aβ aggregates. (4) Senescent microglia instead secrete these failed, phagocytosed products, seeding further aggregates and creating neuritic amyloid plaques corresponding to Braak staging and clinical LOAD progression. Finally, this combination of senescent glia, reduced homeostatic glia support, and non-senescent, inflammatory glial states drive synaptic loss and cognitive decline in LOAD. Colour shading denotes healthy cognition (blue) transitioning into more severe degrees of LOAD progression (purple).

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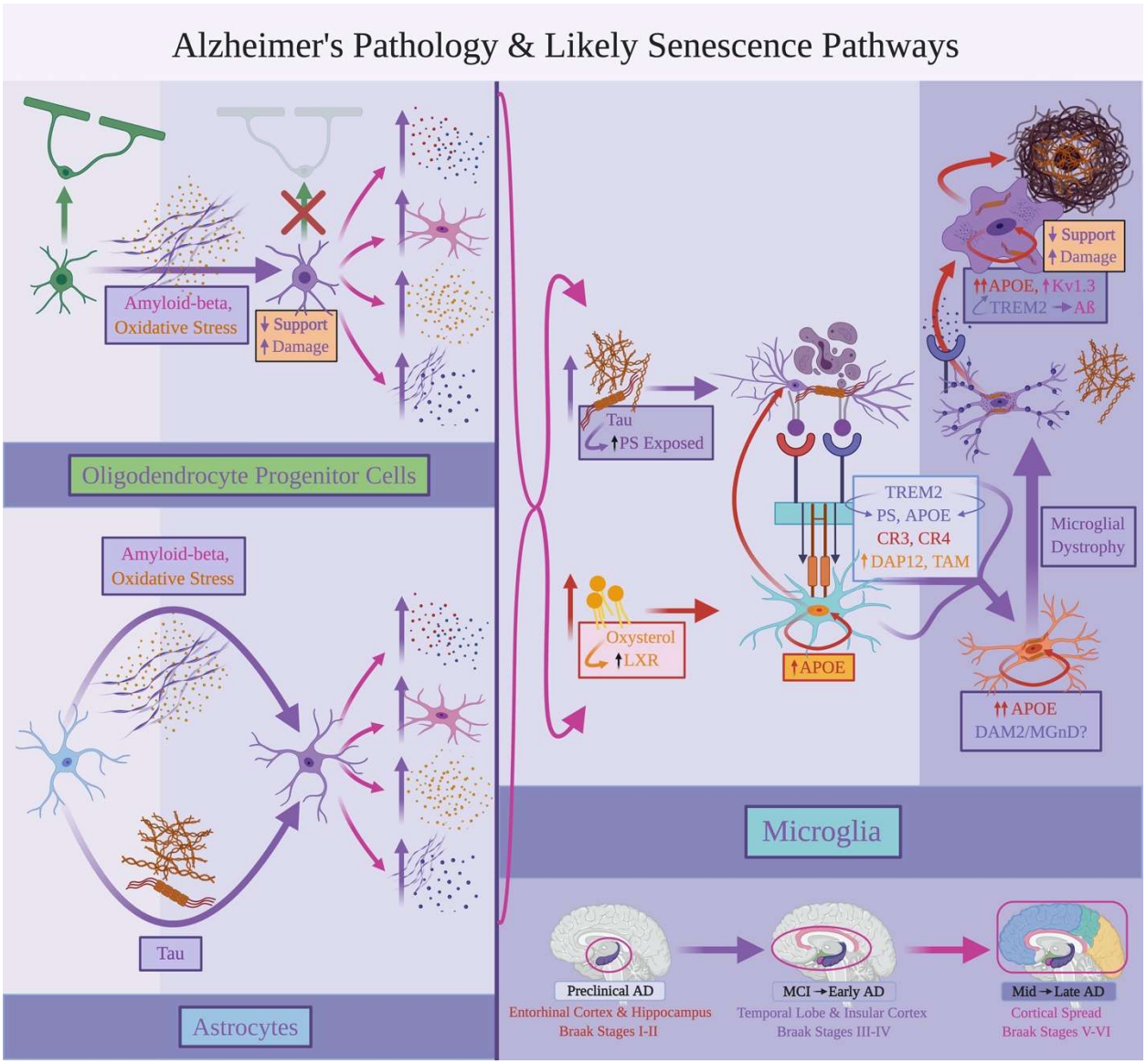

Fig. 3: Alzheimer's Pathology & Likely Senescence Pathways. (Left) Oligodendrocyte progenitor cells (OPCs) and astrocytes both react with amyloid-beta (Aβ) to become senescent; oxidative stress at least indirectly accelerates this senescence induction. Senescent OPCs and astrocytes enhance increasing levels of main LOAD pathologies, ongoing pro-inflammatory microglial states, oxidative stress, and Aβ accumulation that eventually favours conditions that induce overwhelming microglial senescence. (Right) In LOAD, Aβ pathology contributes to oxidative stress that oxidizes cholesterol to form oxysterols. Homeostatic microglia likely utilize liver-X-receptor (LXR) to bind with oxysterols, resulting in upregulated apolipoprotein (APOE) expression. This leads to both increased Aβ phagocytosis and degradation.
Simultaneously, oxidative stress and tau aggregation induce neurons to translocate and expose outer phosphatidylserine (PS). APOE-upregulated microglia in homeostatic states bind PS using various receptors, and perform "phagoptosis" to phagocytose and prematurely kill PS-exposed neurons. Engulfing hyperphosphorylated tau turns microglia senescent and hypofunctional, rendering them unable to properly phagocytose LOAD pathology. Senescent microglia initially secrete failed, phagocytosed products that induce paracrine microglial senescence and form

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neuritic plaques; as decreasing homeostatic microglia are available to phagocytose Aβ, Aβ aggregation continuously accumulates and likely initiates a type I interferon response in senescent microglia. This Aβ-induced response causes *APOE* and *KCNA3* upregulation in senescent microglia, where resulting Kv1.3 protein causes increased, pro-inflammatory cytokine release. Senescent microglia then undergo dystrophy, after adopting a pre-senescent (hyper)-ramified morphology. When engulfing and attempting to contain Aβ and hp-tau aggregates, senescent, dystrophic microglia likely take on a hypertrophic appearance. These "amoeboid" appearing microglia secrete failed aggregates to form neuritic plaques, and negligently permit further neurofibrillary tangle formations in nearby neurons.

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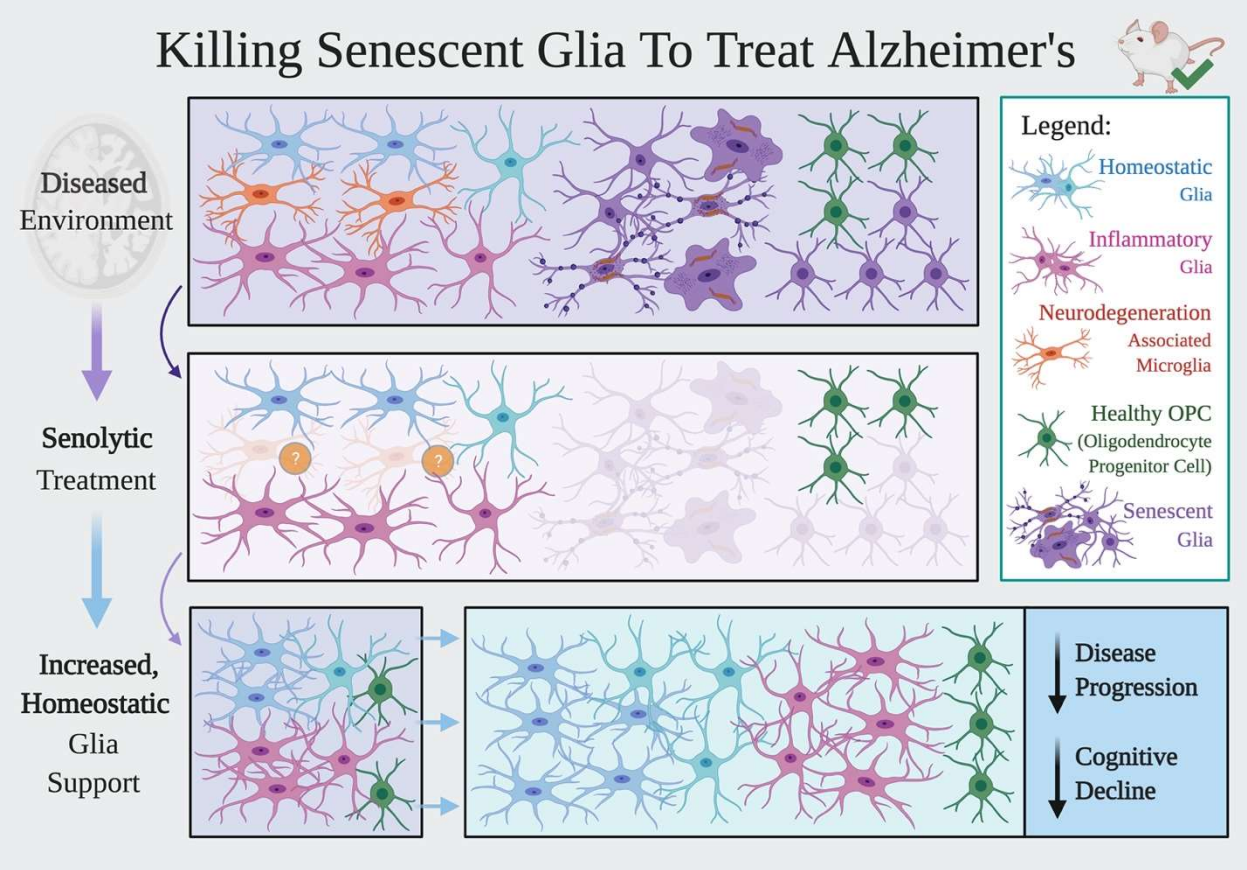

Fig. 4: Killing Senescent Glia To Treat Alzheimer's. Senolytics are drugs that selectively kill senescent cells, and have shown to reduce cognitive decline in disease mouse models. If senescent glia cause irreversible synaptic loss and accumulation of main LOAD pathologies, senolytics should likely treat AD. Remaining glial populations can repopulate and renew homeostatic function to effectively clear out and reduce main LOAD pathologies, without senescent glia detrimentally exerting a gradient of LOAD pathology accumulation. Resulting plasticity should hopefully return the aging brain back to a healthy cognition or preclinical LOAD state, or at least halt further LOAD progression. Future treatments can also be utilized in combination with senolytics. This would allow better restoring of glial homeostatic functions, and minimize damage accrued by pro-inflammatory glial states and other LOAD pathologies. It is unknown if neurodegeneration or disease-associated microglial phenotypes are targeted by senolytics.

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