

Perspective

# Alzheimer's disease: From immunotherapy to immunoprevention

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## SUMMARY

Recent A $\beta$ -immunotherapy trials have yielded the first clear evidence that removing aggregated A $\beta$  from the brains of symptomatic patients can slow the progression of Alzheimer's disease. The clinical benefit achieved in these trials has been modest, however, highlighting the need for both a deeper understanding of disease mechanisms and the importance of intervening early in the pathogenic cascade. An immunoprevention strategy for Alzheimer's disease is required that will integrate the findings from clinical trials with mechanistic insights from preclinical disease models to select promising antibodies, optimize the timing of intervention, identify early biomarkers, and mitigate potential side effects.

## INTRODUCTION

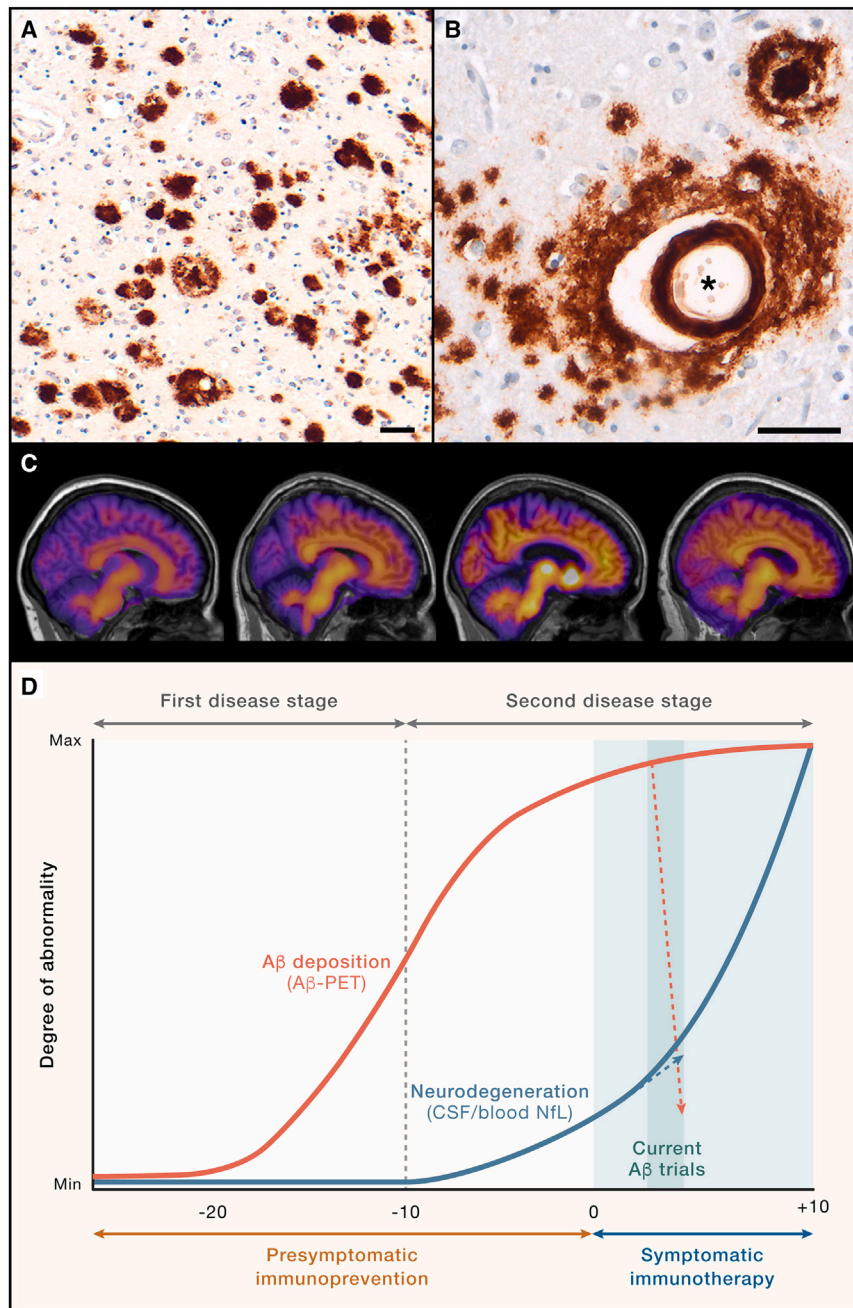
The recent reports that monoclonal antibodies (lecanemab [Lecanemab] and donanemab) stimulate the removal of abnormal  $\beta$ -amyloid (A $\beta$ ) from the brain and slow the progression of early Alzheimer's disease (AD)<sup>1,2</sup> have given the research community the first clear clinicopathological indication that a disease-modifying treatment for AD is feasible. Together with evidence that another monoclonal antibody (aducanumab [Aduhelm])<sup>3</sup> also may be beneficial, the results provide clinical support for the importance of aberrant A $\beta$  in the pathogenesis of AD. The findings also strengthen the “amyloid (A $\beta$ ) cascade” hypothesis, which holds that the seminal event in the ontogeny of AD is the misfolding and aggregation of A $\beta$ , followed by a host of sequelae that comprise the full clinical and pathological phenotype of the disease.<sup>4,5</sup>

Although there is now renewed hope for disease-modifying therapies, it is important to caution that the clinical benefit of the antibodies in the trials was limited and that the disease still progressed in treated subjects, albeit at a slower pace. Removal of aberrant A $\beta$  in symptomatic AD is unlikely to be a cure for the disease, which begins to germinate in the brain 20–30 years before the onset of obvious cognitive impairment.<sup>6–8</sup> By the time the signs and symptoms of AD first appear clinically, damage to the brain is considerable and at least partially beyond repair; hence, a full return to baseline functionality is unlikely, in line with the limited efficacy of the antibody treatment trials. Hence, a prevention strategy is essential; chronic degenerative diseases such as AD are most effectively treated as early in their development as possible, preferably well before they become symptomatic.<sup>9,10</sup> Lessons learned from immunization treatment trials, combined with mechanistic insights from experimental and biomarker investigations, have now brought us a step closer to this goal.

## THE PATHOBIOLOGY OF AD

AD is defined histopathologically by the profusion of two proteinaceous lesions in the brain—A $\beta$  plaques and neurofibrillary (Tau) tangles. Burgeoning evidence indicates that the A $\beta$  and Tau proteins misfold, self-assemble, and propagate by an endogenous mechanism closely resembling the seeded aggregation and spread of the prion protein (PrP) in Creutzfeldt-Jakob disease and other prionopathies.<sup>11–14</sup> A $\beta$  plaques disrupt circuits and neighboring brain cells,<sup>15–17</sup> but A $\beta$  also forms small, soluble, oligomeric assemblies that impair the function of neurons and glia.<sup>18,19</sup> In addition, A $\beta$  often accumulates in the walls of small to medium sized cerebral blood vessels, manifesting as cerebral A $\beta$  angiopathy (CAA)<sup>20</sup> (Figure 1). Although the amount of CAA varies widely among AD patients, nearly half of end-stage AD patients exhibit moderate-to-severe CAA.<sup>21,22</sup>

A $\beta$  plaques and tau tangles are both abundant in advanced AD, but genetic, pathologic, and biomarker findings show that A $\beta$ -proteopathy is the crucial early impetus for the disease; widespread tauopathy and other sequelae are essential drivers of behavioral impairment that are downstream of A $\beta$ .<sup>7,27,28</sup> AD thus is thought to progress in two stages; the first stage is characterized by the emergence and seeded propagation of aberrant A $\beta$  and A $\beta$ -associated pathologies, and the second stage includes a complex assortment of secondary changes that include tangles, inflammation, vascular abnormalities, and neurodegeneration.<sup>29</sup> In the second stage, the disease appears to become at least partially independent of A $\beta$  deposition<sup>24–26</sup> (Figure 1). This bi-phasic trajectory of AD pathogenesis has important implications for both treatment and prevention strategies. As a defining pathologic feature of AD, tauopathy also has been the object of immunization strategies,<sup>30,31</sup> but the pathogenic primacy of A $\beta$  makes it a particularly attractive target for early prevention.



**Figure 1. The pathologic progression of AD, immunoprevention, and the impact of immunotherapy in symptomatic patients**

(A and B) Immunohistochemical detection of Aβ deposition in AD brain as plaques (A) and cerebral β-amyloid angiopathy (CAA; black asterisk in B); the affected vessel is surrounded by diffuse parenchymal Aβ deposits, and a dense-core plaque is in the upper right. CAA is moderate-to-severe in nearly half of all AD cases, and CAA has been linked to the side effects of Aβ-immunotherapy; antibody 4G8, Nissl counterstain; scale bars are 50 μm.

(C) Representative Aβ-PET images (left to right) from a control person (non-mutation carrier), a mutation carrier about 10 years before symptom onset, and two mutation carriers that are symptomatic (Pittsburgh compound B [PiB] tracer; shown are participants with familial AD<sup>23</sup>). The increase in PiB retention primarily occurs in the presymptomatic phase.

(D) In the two-stage model of AD,<sup>24–26</sup> the first stage is dominated by Aβ deposition. The second stage commences approximately 10 years before symptom onset and becomes partly independent of Aβ deposition with the emergence of clear signs of neurodegeneration (as assessed, e.g., by NfL levels in CSF or blood) and, eventually, behavioral impairments.<sup>26</sup> Targeting aberrant Aβ as immunoprevention (prevention of the disease) is likely to be most successful when initiated during or prior to the first stage. Given the growing pathologic complexity of the disease, it is not clear how much clinical benefit can be expected from Aβ-removing therapies alone beyond this time point. Indeed, Aβ-immunotherapy trials for 18 months with aducanumab, lecanemab, or donanemab removed >60% of the deposited Aβ, but NfL continued to rise (albeit at a reduced pace), paralleling the slowed—but not stopped—cognitive decline in treated subjects.

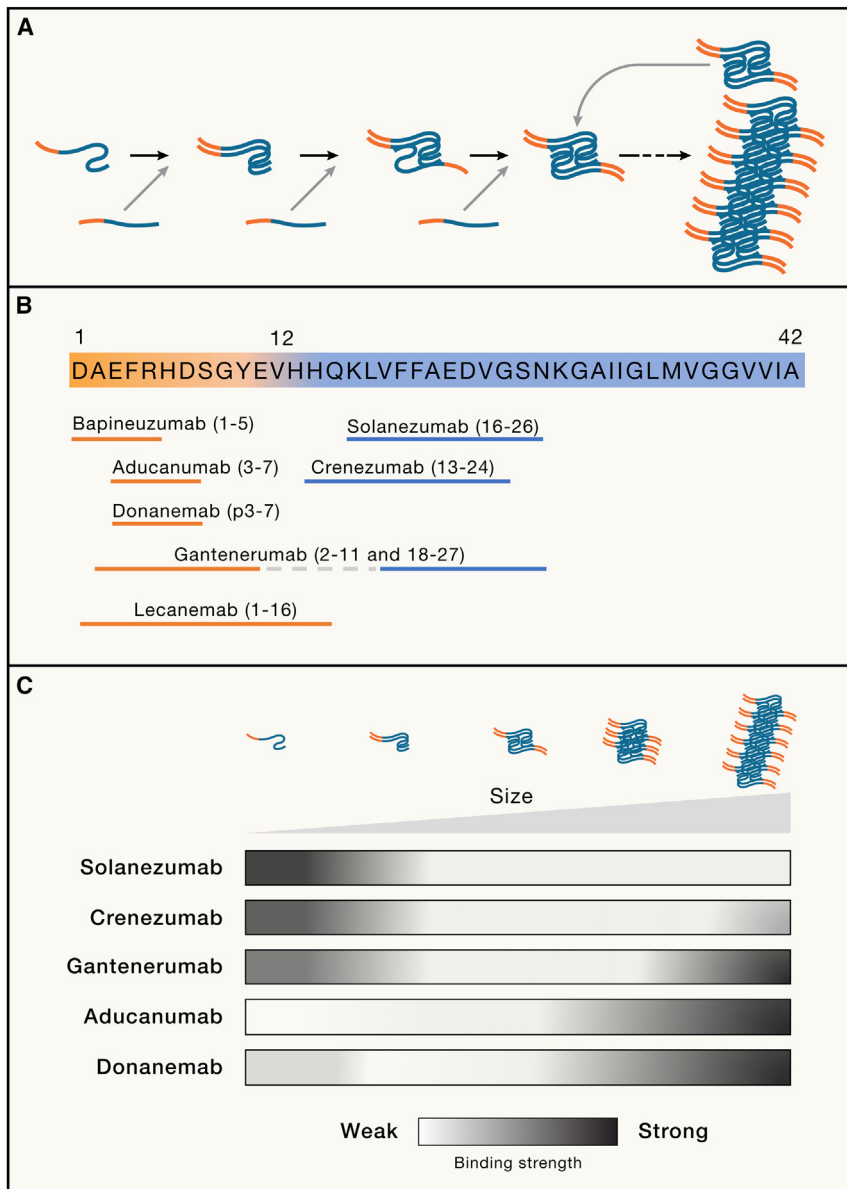
decline,<sup>35</sup> along with fairly compelling evidence in postmortem tissue for the clearance of Aβ plaques.<sup>36,37</sup> However, the meningoencephalitic side effects, coupled with the inability to fully reverse the errant immune response, was a setback for active immunization. As a result, much research was steered toward passive immunotherapy with humanized monoclonal antibodies as a potentially safer alternative.

The antibodies that have advanced the farthest in clinical development include bapineuzumab, solanezumab, crenezumab,

gantenerumab, aducanumab, lecanemab, and donanemab (Figure 2). These antibodies recognize partly different antigenic sites on Aβ, and they differ in their apparent clinical efficacy and in their ability to lower plaque load. All of them have been tested in phase 3 studies of patient cohorts with mild cognitive impairment or mild AD dementia.<sup>2,38</sup> Except for solanezumab and crenezumab, the antibodies reduced cerebral Aβ content, as measured by positron-emission tomography (Aβ-PET).<sup>2,28</sup> Although postmortem confirmation is largely lacking, based on previous comparisons of the Aβ-PET signal and postmortem Aβ load, a corresponding reduction of Aβ deposits is likely.<sup>37,39</sup>

## Aβ IMMUNOTHERAPY FOR AD

Immunization therapy for AD was launched in earnest in 1999, when active immunization of Aβ-precursor protein (APP) transgenic mice with synthetic polymers of Aβ was shown to dramatically reduce cerebral plaque burden.<sup>32</sup> This report prompted a flurry of research on the potential of immune mechanisms to treat or prevent AD.<sup>33</sup> The initial clinical trial of active Aβ immunotherapy in humans (AN1792) was halted when a subset of the recipients developed aseptic meningoencephalitis.<sup>34</sup> A follow-up study of a small number of patients showed hints of slowed cognitive



**Figure 2. A $\beta$  aggregation and the epitopes recognized by therapeutic antibodies**

(A) A $\beta$  aggregation starts with a slow nucleation phase during which A $\beta$  assumes an alternative conformation that converts and binds to other A $\beta$  molecules to form the initial segment of the amyloid fibril. With increasing length, the growing fibril eventually breaks and releases seeding-active A $\beta$  multimers, at which stage the process becomes self-propagating (based on Jucker and Walker<sup>11</sup>). As deposition progresses, A $\beta$  comprises a mixture of multimers, ranging from small soluble oligomers to long amyloid fibrils, which differ in their cytotoxicity and ability to seed further aggregation.<sup>18,19,40,41</sup> The growing amyloid fibril schematically depicted here consists of two twisted protofilaments (based on Yang et al.<sup>42</sup> for brain-derived A $\beta$ 42 fibrils). Note that the N-terminal amino acids (orange) are exposed from the hydrophobic amyloid core (blue).

(B) Diagram of A $\beta$ 42 showing the amino acid epitopes that therapeutic antibodies are thought to recognize (based on Plotkin and Cashman<sup>43</sup>). Common to the antibodies that cleared A $\beta$  deposits in clinical trials (gantenerumab, aducanumab, donanemab, lecanemab) is that they recognize N-terminal amino acids (orange), i.e., epitopes that are exposed on mature amyloid fibrils. In contrast, solanezumab and crenezumab only recognize mid-sequence epitopes that are buried within the amyloid fibril; hence, these antibodies mainly recognize monomeric A $\beta$ .

(C) Schematic illustration of the binding strength of five different antibodies to A $\beta$  that was derived from native amyloid-laden brain samples (AD and mouse models) and fractionated according to size. The two antibodies that most effectively remove A $\beta$  from the brain (donanemab, aducanumab) recognize predominantly large amyloid aggregates (based on Uhlmann et al.<sup>44</sup>).

more slowly than in controls, thereby mirroring the slowed (but not stopped) decline of cognitive changes<sup>1,2,45,46,48</sup> (Figure 1).

Unfortunately, in some immunotherapy patients, the removal of aggregated A $\beta$  has been associated with troublesome and sometimes serious side effects known as amyloid-related imaging abnormalities (ARIA),<sup>1-3,45</sup> which appear

to be linked to the abundance of pre-existing A $\beta$  deposition, especially as CAA. A $\beta$  removal has been associated with an expansion of ventricular volume and an increased reduction of brain volume,<sup>2,52,53</sup> the functional significance of which remain uncertain. Overall, both the limited clinical benefit of antibody therapy and the risk of serious side effects that are associated with the presence of a high amyloid burden underscore the importance of starting treatment much earlier in the pathogenic process.

#### FROM IMMUNOTHERAPY TO IMMUNOPREVENTION: WHAT DO WE NEED?

Below, we consider four key research objectives that are needed to extend the results of past and ongoing clinical trials of anti-A $\beta$

Thus far, the antibodies that yielded the greatest removal of A $\beta$  deposition (>60% after 18 months of treatment)—lecanemab, donanemab, and aducanumab—have shown evidence of slowed clinical decline,<sup>1-3</sup> though it is important to stress that direct comparison of clinical efficacy is hindered by differences in the trials, such as dosage, treatment schedule, and the patient populations evaluated.

Notably, reduction of A $\beta$  burden was accompanied by decreased phosphorylated Tau (pTau) species and glial fibrillary acidic protein (GFAP) in the cerebrospinal fluid (CSF) or blood.<sup>1,2,45-48</sup> Thus, immunotherapy not only decreased A $\beta$  load but (based on fluid biomarkers) may also have decreased cerebral A $\beta$ -associated tauopathy and astrocytic activation. In contrast, neurofilament light chain (NfL; a marker of neuronal abnormalities)<sup>49-51</sup> continued to rise in treated subjects, albeit somewhat

antibodies to the effective immunoprevention of AD: define the best molecular target for A $\beta$ -immunotherapy (epitopes), optimize the schedule of treatment (timing), establish early biological indicators of preventive efficacy (biomarkers), and identify and mitigate potential adverse reactions to antibody administration (side effects).

### Identify the optimal A $\beta$ epitopes

Based on the results of the recent clinical trials, the most coherent (but still provisional) conclusion is that lowering cerebral A $\beta$  load can slow the progression of AD. Solanezumab selectively binds A $\beta$  monomers; although a meta-analysis suggests that it may have had some clinical efficacy,<sup>54</sup> solanezumab failed to reach primary endpoints in clinical trials.<sup>55</sup> Monomeric A $\beta$  is abundant in the brain, and its complete neutralization would require stoichiometric amounts of high-affinity antibodies able to compete with the binding of monomers to existing A $\beta$  aggregates. Preclinical evidence indicates that the toxicity of A $\beta$  is linked to its aggregated state,<sup>26,27</sup> and the antibodies that have shown the best evidence of clinical efficacy also achieved the largest reduction of aggregated A $\beta$ .<sup>1–3</sup> For these reasons, an antibody that generally recognizes monomeric A $\beta$  is unlikely to be the most favorable immunotherapeutic tool.

The multiple manifestations of aberrant A $\beta$  could present challenges for immunoprevention. A $\beta$  multimers range in size from small oligomers to protofibrils and long amyloid fibrils, and they differ in their cytotoxicity and ability to seed further aggregation (Figure 2). Moreover, the predominant species of A $\beta$  can vary among patients, between the vasculature and parenchyma, and over the course of the disease.<sup>12,20,42,56,57</sup> Given the biochemical and structural complexity of A $\beta$  aggregates, the best epitopic targets for the prevention or removal of A $\beta$  multimers remain uncertain. As one example, the positive clinical outcome of the lecanemab trial might imply that A $\beta$ -protofibrils are a particularly promising target.<sup>58</sup> Lecanemab was raised against recombinant “arctic” mutant (E22G) A $\beta$ , a form of the protein that is linked to a rare familial form of AD characterized by marked accumulation of protofibrils.<sup>59</sup> However, the protofibrillar nature of arctic A $\beta$  in patients’ brains is incompletely understood,<sup>60</sup> and there is evidence that recombinant A $\beta$  folds into a conformation that differs from that of A $\beta$  that folds within the brain.<sup>57,42,60</sup> Hence, the clinical efficacy of lecanemab<sup>1</sup> could result from the overall reduction of A $\beta$  (at which the antibody is quite effective) and not from the neutralization of a specific type of multimer (i.e., protofibrils).

Similarly, *in vitro* studies suggest that aducanumab decreases A $\beta$  oligomer generation from secondary nucleation,<sup>61</sup> but the general reduction of A $\beta$  load in the aducanumab clinical trial<sup>3</sup> precludes linking the clinical outcome to certain oligomeric species. To complicate things further, smaller, “soluble” assemblies might be in a state of dynamic equilibrium with A $\beta$  plaques,<sup>18</sup> such that eliminating plaques would indirectly reduce the population of oligomers and protofibrils, and vice versa (Figure 2). Imaging and fluid biomarkers for oligomeric A $\beta$  are needed to meaningfully connect pathogenic molecular species to clinical efficacy (see [optimize biomarker use](#) below).

Pyroglutamate-modification of A $\beta$  (e.g., A $\beta$ <sub>N3pE</sub>) enhances the propensity of the protein to aggregate; A $\beta$ <sub>N3pE</sub> emerges predom-

inantly in later stages of cerebral  $\beta$ -amyloidosis<sup>62,63</sup> and thus exemplifies how abnormal A $\beta$  can change over the course of AD. Donanemab is directed at A $\beta$ <sub>N3pE</sub>, and it has been shown to be highly effective at removing amyloid in symptomatic patients.<sup>2</sup> However, it is conceivable that donanemab’s specificity for a relatively late-arising epitope could diminish its ability to impede A $\beta$  deposition at a much earlier stage of disease. Another molecular modification that occurs late in the maturation of A $\beta$  plaques is phosphorylation at position 8 (A $\beta$ <sub>pS8</sub>).<sup>64</sup> Accordingly, A $\beta$ -immunotherapy might need to be tailored to the characteristics of A $\beta$  at different stages of AD to achieve optimal therapeutic and preventive efficacy (see [establish the best timing for immunoprevention](#) below).

To identify the most promising immunotherapeutic or immunopreventive A $\beta$  epitope, clinical studies directly comparing several different antibodies would be informative. Moreover, postmortem biochemical analyses of the brain after treatment will then help to pinpoint changes induced by the antibodies that are most pertinent to effective prevention. In parallel, comparison of antibodies and the multimers they engage in preclinical models,<sup>65</sup> along with structural studies of the epitopes recognized by the various antibodies, are needed. Together with such properties of the antibodies as affinity, immunoglobulin subtype, posttranslational modifications, and half-life in blood (as well as the inherent immunogenicity of the antibodies themselves<sup>66</sup>), these data should help to facilitate the design of next-generation antibodies and define the most suitable molecular target for immunoprevention.

### Establish the best timing for immunoprevention

Abnormal A $\beta$  begins to accumulate in the brain two to three decades before the clinical signs and symptoms of AD become manifest.<sup>6–8</sup> True primary prevention—stopping A $\beta$  aggregation before it begins—is a particularly attractive objective, but establishing an acceptable risk:benefit ratio for long-term administration of a preventive agent is a formidable task. From a practical standpoint, secondary prevention is a more likely scenario, i.e., initiating preventive measures in response to biomarker evidence that aberrant A $\beta$  has begun to accumulate, but before the onset of the cognitive and behavioral changes of AD<sup>67</sup> (Figure 1).

In the two-stage model of AD,<sup>24–26</sup> A $\beta$  proteopathy initially drives the disease, but its relative influence diminishes concomitant with the emergence of myriad subsequent changes that include neurofibrillary tangle formation, inflammation, neurodegeneration, and, eventually, behavioral impairments.<sup>5</sup> The transition from the first to the second stage is heralded by a steep rise in the CSF levels of NfL, and this has been estimated to occur around 10 years before the onset of symptoms.<sup>26</sup> At least in mouse models, the increase in NfL (and thus presumed neurodegeneration) coincides with the saturated A $\beta$  seeding activity of brain tissue.<sup>26</sup> Once the second stage is underway, it is not clear how much clinical benefit can be expected from A $\beta$ -removing therapies alone (Figure 1); rather, treatments directed at both A $\beta$ -proteopathy and its sequelae may be required. A clinical trial targeting A $\beta$  in the first disease stage is being planned in carriers of dominant AD mutations 10 years or more prior to the estimated onset of symptoms (<https://clinicaltrials.gov/ct2/show/NCT05552157>). In addition, a clinical trial targeting both A $\beta$  (lecanemab) and Tau (antibody

E2814) in the second disease stage (i.e., less than 10 years from the estimated disease onset) was recently launched (<https://clinicaltrials.gov/ct2/show/NCT05269394>).

For future investigations aiming to optimize the timing of immunoprevention, we need to determine whether the antibodies must be given continuously or whether intermittent administration will suffice, and what the frequency and duration of treatment should be. When, and how often, treatment should be repeated for maximal efficacy in humans is not yet known, but insights into such mechanistic questions can be gleaned from studies in animal models. For example, experiments with mouse models suggest that acute removal of A $\beta$  seeds at a very early stage of A $\beta$  deposition delays both the accumulation of A $\beta$  and the onset of downstream pathologies later in life.<sup>68,44</sup> Thus, it might not be necessary to continuously administer anti-A $\beta$  antibodies to delay or prevent A $\beta$  deposition (and, by extension, AD). Finally, we need to determine whether methods to augment antibody entry into the brain, such as brain shuttle antibody constructs<sup>69</sup> or ultrasound,<sup>70</sup> are necessary (or even desirable) to reduce the number of treatments required for immunoprevention.

### Optimize biomarker use

Advances in A $\beta$ -PET imaging and in the measurement of A $\beta$  in biofluids have substantiated the decades-long presymptomatic development of AD,<sup>6,71,72</sup> and these technologies have been foundational for the implementation of recent clinical trials.<sup>71,72</sup> As sensitive and informative as these methods have become, they do not detect aberrant A $\beta$  that might be revealed even earlier if the brains were to be analyzed with sensitive biochemical or immunohistological techniques.<sup>44,73–75</sup> Similarly, when A $\beta$ -PET scans signal that immunotherapy has reduced the A $\beta$ -load below detection levels, it is likely that a pathologically significant amount of aberrant A $\beta$  remains. Moreover, current A $\beta$ -PET imaging and biofluid A $\beta$  measurements do not provide detailed information about the biochemical and structural characteristics of cerebral A $\beta$  before and after immunotherapy, nor can they satisfactorily discriminate vascular from parenchymal amyloid to gauge the risk of side effects (see [mitigate the side effects](#) below). Thus, further improvements in assay sensitivity and specificity for early and different forms of aberrant brain A $\beta$  are needed.

Multiple biomarkers in fluids now can track many of the sequelae of A $\beta$  proteopathy. Major progress has been achieved in measuring A $\beta$ -associated pTau (pTau181, pTau217, pTau231) in CSF and blood.<sup>76,77</sup> More recently, tests have been developed to detect activated astrocytes and microglia (GFAP and soluble triggering receptor expressed on myeloid cells 2 [Trem2], respectively).<sup>72,78,79</sup> In presymptomatic AD, the trajectories of these biomarkers closely parallel those of A $\beta$ -deposition. In contrast, NfL in the CSF increases robustly only after half-maximal A $\beta$  deposition is reached, and this appears to mark the transition from the first to the second stage of AD progression<sup>26</sup> (Figure 1).

Despite the considerable clinical utility of fluid biomarkers, the interpretation of clinical trial outcomes would be enriched by a better understanding of the molecular and cellular alterations that these biomarkers represent. For example, the paradoxical

finding that pTau fluid biomarkers are strongly associated with the trajectories of A $\beta$ -deposition but poorly with aberrant Tau, as measured by PET, currently lacks a persuasive explanation. In addition, the pathophysiologic basis of increased GFAP and Trem2 levels in biofluids is still uncertain, as is the means by which proteinaceous biomarkers, in particular intracellular structural proteins such as Tau, GFAP, and NfL, make their way into the CSF and blood.

Many mechanistic questions about biomarkers now can be addressed in disease models (mainly genetically modified mice) that enable a direct and timely comparison of brain pathology to protein changes in biofluids. Animal models can also help to clarify issues that confound investigations of humans, including diagnostic uncertainty, comorbidities, perimortem irregularities (such as agonal state and postmortem interval), and lifestyle variability. For example, in APP-transgenic mouse models, A $\beta$  deposition per se (i.e., in the absence of neurofibrillary tangles and neuron loss) is sufficient to induce increases in CSF pTau.<sup>80</sup> Moreover, transgenic mouse models manifesting distinct proteopathies enable the separation of A $\beta$ -dependent secondary biomarker changes from those associated with possible comorbid pathologies such as  $\alpha$ -synucleinopathy.<sup>81</sup>

Finally, to optimize immunopreventive strategies, biomarkers of early pathogenesis with a robust effect size are needed to define both the inception and initial trajectory of the disease process. For instance, in both prion diseases<sup>14</sup> and AD,<sup>26</sup> the respective seeding activities of PrP and A $\beta$  in brain tissue rise steeply in the initial stage of protein aggregation before reaching a plateau around the time that neurodegeneration becomes apparent. Seeds of misfolded PrP have been amplified from the CSF of patients with Creutzfeldt-Jakob disease,<sup>82</sup> and  $\alpha$ -synuclein seeds have been detected in CSF and blood of patients with  $\alpha$ -synucleinopathies.<sup>83,84</sup> Similar novel biomarkers would help to characterize the earliest stages of AD and could serve as informative readouts in immunoprevention trials.

### Mitigate the side effects

ARIAs can be a serious side-effect of A $\beta$  immunotherapy.<sup>1,2,85</sup> ARIAs include focal cerebral edema/effusion (ARIA-E) and hemosiderin accumulation (ARIA-H, a marker of previous microbleeds).<sup>20,86</sup> Although ARIAs are often asymptomatic and can be managed in treated subjects by titration of the antibody dose,<sup>1,85,87</sup> in some instances severe reactions to treatment have occurred, raising concerns about the risk:benefit ratio of immunotherapy for AD.<sup>38,88,89</sup>

The mechanisms underlying ARIAs are incompletely understood, but the abnormalities are strongly associated with the presence of CAA and associated changes.<sup>20,90</sup> CAA appears to be increased—at least temporarily—in response to A $\beta$  immunotherapies that reduce parenchymal A $\beta$  both in mouse models<sup>91</sup> and in humans.<sup>36,37,92,93</sup> This increase may result from the translocation of A $\beta$  from the parenchyma to the vascular wall,<sup>94,95</sup> although the precise mechanism is uncertain. As the immune system (e.g., perivascular macrophages) engages with A $\beta$  in the vascular wall, the blood vessel is compromised and becomes prone to leakage and rupture.<sup>20</sup> In rare cases, A $\beta$ -immunotherapy combined with blood thinners has caused fatal

cerebral hemorrhage in subjects with CAA,<sup>88</sup> an adverse event that could have been predicted from preclinical studies in mouse models.<sup>91,96</sup>

ARIAs are most frequent in immunotherapy recipients bearing the  $\epsilon 4$  allele of the gene for apolipoprotein E ( $APOE\epsilon 4$ ),<sup>20,38</sup> consistent with  $APOE\epsilon 4$  being a prominent risk factor for CAA.<sup>97</sup> (CAA can also be significant in non-carriers of  $APOE\epsilon 4$ ,<sup>98</sup> albeit less commonly than in carriers.) Because CAA is moderate-to-severe in approximately 45% of AD cases,<sup>21,22</sup>  $A\beta$ -immunotherapy could put nearly half of symptomatic AD patients at greater risk of ARIAs. Experimental work with mouse models has found that  $A\beta$ -immunotherapy-related hemorrhages are evident only when substantial CAA is present.<sup>91,99</sup> It is therefore possible that the risk of ARIAs would be diminished or even eliminated if  $A\beta$  antibodies were administered before CAA became widespread in the brain, i.e., as a preventive measure.

The incidence of treatment-related ARIAs differed among past therapeutic trials of anti- $A\beta$ -monoclonal antibodies.<sup>38,86</sup> It is uncertain whether this variation is related to characteristics of the antibodies (such as their antigen-recognition profiles), to differences in the trial participants (such as their disease stage or CAA load), or to methodological issues such as sensitivity in ARIA detection. CAA can be suspected based on certain clinical and biomarker signs,<sup>20,100</sup> but a definitive biomarker for CAA would enhance the prognostic precision for ARIAs in potential recipients of immunotherapeutics. CAA-specific PET ligands could emerge from recent findings that the three-dimensional architecture of  $A\beta$  fibrils in CAA differs from that in plaques.<sup>57,42</sup> The discovery that the protein medin co-deposits exclusively with  $A\beta$  in the vasculature<sup>101</sup> could also enable the development of a biomarker specific for CAA.

Additional research is needed to delineate the fundamental mechanisms underlying the CAA-associated side effects of  $A\beta$ -immunotherapy. Recent insights into the clearance of  $A\beta$  by the vasculature-associated brain fluid drainage system,<sup>102,103</sup> the role of perivascular macrophages,<sup>104</sup> and neuro-immune interactions along CAA-prone meningeal and parenchymal blood vessels<sup>105</sup> have set the stage for further research in preclinical models to understand and mitigate the side effects of  $A\beta$ -immunotherapy. This work is also important for gauging any possible risks posed by immunopreventive measures initiated before CAA becomes widespread in the brain.

## CONCLUDING REMARKS

Evidence of disease modification by monoclonal antibodies is a small but encouraging step forward in the campaign to subdue AD. We now need to extend the lessons learned from treatment trials to a new framework for the prevention of the disorder. A salient challenge for implementing early prevention is the need for robust prognostic indicators of incipient disease, whereas later preventive measures will be hindered by the relative complexity of advanced disease, which may necessitate combination therapy for multiple targets.<sup>10,106</sup>

To learn as much as possible from current and past treatment trials, an increasingly refined analysis is essential, not only of the behavioral and biomarker findings during the first 18 months of

treatment but, more importantly, also of the biomarker trajectories beyond this point. This applies to participants who continue antibody treatment past 18 months and those who discontinue treatment at any point in the trial. Ideally, such an analysis should be combined with a careful investigation of the biochemical and pathological status of the brain postmortem (similar to the long-term follow-up examination of actively immunized subjects<sup>37</sup>).

More research with experimental disease models is needed to address fundamental questions that have emerged from the immunotherapy trials.<sup>65</sup> For example, the interpretation of biomarker trajectories and treatment responses in humans is hampered by the incomplete mechanistic understanding of what these changes represent in the brain. Advances in analytic technologies now enable the measurement of low-abundance proteins in very small volumes of biofluids, making it feasible to measure the same analytes in the CSF and blood of mice and humans; such comparative analyses can inform the design and interpretation of therapeutic and prevention trials.<sup>26,49,80,107</sup> More broadly, the development of advanced disease models that more completely represent the complexity of later-stage AD would improve the translatability of basic research.<sup>65</sup>

Although passive immunization with anti- $A\beta$  antibodies is especially promising as a preventive approach to AD, it is useful to consider alternative strategies. For instance,  $A\beta$ -proteopathy might be prevented indirectly by targeting proteins that co-deposit with  $A\beta$  (e.g., ApoE<sup>108</sup> or  $\beta_2$ -microglobulin<sup>109</sup>), or by delivering antibodies against Trem2, which stimulate microglial phagocytosis of  $A\beta$  assemblies.<sup>110,111</sup>

There are also good reasons to keep active immunization in play; current practical impediments to passive antibody treatment, such as cost and the mode and frequency of delivery, are likely to limit the widespread deployment of passive immunoprevention. The chief drawbacks of active immunization (vaccination) are the risks associated with an immune reaction to a “self” antigen and the difficulty of moderating an errant immune response.<sup>112</sup> Since the termination of the pioneering  $A\beta$  vaccine trial,<sup>113</sup> basic research has advanced the safety and efficacy of active immunization.<sup>114,115</sup> Although considerable challenges remain, if these can be overcome, active immunization could become a relatively simple, inexpensive, and accessible immunopreventive measure.

Finally, it should be emphasized that dementia can result from pathologies such as primary tauopathy,  $\alpha$ -synucleinopathy, TDP-43 proteopathy, vascular disease,<sup>106,116</sup> and many others. In older people, several of these degenerative processes can be comorbid with AD.<sup>29</sup> Prevention of AD per se thus will not completely eliminate the risk of dementia. Even so, because AD is the most common cause of dementia,<sup>117</sup> effective prevention would have considerable benefit for the world's increasingly long-lived population.

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#### DECLARATION OF INTERESTS

The authors declare no competing interests.

#### REFERENCES

- van Dyck, C.H., Swanson, C.J., Aisen, P., Bateman, R.J., Chen, C., Gee, M., Kanekiyo, M., Li, D., Reyderman, L., Cohen, S., et al. (2023). Lecanemab in early Alzheimer's disease. *N. Engl. J. Med.* 388, 9–21. <https://doi.org/10.1056/NEJMoa2212948>.
- Sims, J.R., Zimmer, J.A., Evans, C.D., Lu, M., Ardayfio, P., Sparks, J., Wessels, A.M., Shcherbinin, S., Wang, H., Monkul Nery, E.S., et al. (2023). Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA* 330, 512–527. <https://doi.org/10.1001/jama.2023.13239>.
- Budd Haeberlein, S., Aisen, P.S., Barkhof, F., Chalkias, S., Chen, T., Cohen, S., Dent, G., Hansson, O., Harrison, K., von Hehn, C., et al. (2022). Two randomized phase 3 studies of aducanumab in early Alzheimer's disease. *J. Prev. Alzheimers Dis.* 9, 197–210. <https://doi.org/10.14283/jpad.2022.30>.
- Hardy, J., and Selkoe, D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353–356. <https://doi.org/10.1126/science.1072994>.
- De Strooper, B., and Karran, E. (2016). The cellular phase of Alzheimer's disease. *Cell* 164, 603–615. <https://doi.org/10.1016/j.cell.2015.12.056>.
- Jansen, W.J., Ossenkoppele, R., Knol, D.L., Tijms, B.M., Scheltens, P., Verhey, F.R., Visser, P.J., Amyloid Biomarker Study Group, Aalten, P., Aarsland, D., et al. (2015). Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *JAMA* 313, 1924–1938. <https://doi.org/10.1001/jama.2015.4668>.
- Jack, C.R., Jr., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., Holtzman, D.M., Jagust, W., Jessen, F., Karlawish, J., et al. (2018). NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers. Dement.* 14, 535–562. <https://doi.org/10.1016/j.jalz.2018.02.018>.
- McDade, E., Wang, G., Gordon, B.A., Hassenstab, J., Benzinger, T.L.S., Buckles, V., Fagan, A.M., Holtzman, D.M., Cairns, N.J., Goate, A.M., et al. (2018). Longitudinal cognitive and biomarker changes in dominantly inherited Alzheimer disease. *Neurology* 91, e1295–e1306. <https://doi.org/10.1212/WNL.0000000000006277>.
- Selkoe, D.J. (2012). Preventing Alzheimer's disease. *Science* 337, 1488–1492. <https://doi.org/10.1126/science.1228541>.
- McDade, E., Libre-Guerra, J.J., Holtzman, D.M., Morris, J.C., and Bateman, R.J. (2021). The informed road map to prevention of Alzheimer disease: a call to arms. *Mol. Neurodegener.* 16, 49. <https://doi.org/10.1186/s13024-021-00467-y>.
- Jucker, M., and Walker, L.C. (2013). Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature* 501, 45–51. <https://doi.org/10.1038/nature12481>.
- Jucker, M., and Walker, L.C. (2018). Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases. *Nat. Neurosci.* 21, 1341–1349. <https://doi.org/10.1038/s41593-018-0238-6>.
- Goedert, M. (2015). NEURODEGENERATION. Alzheimer's and Parkinson's diseases: the prion concept in relation to assembled A $\beta$ , tau, and  $\alpha$ -synuclein. *Science* 349, 1255–1255. <https://doi.org/10.1126/science.1255555>.
- Collinge, J. (2016). Mammalian prions and their wider relevance in neurodegenerative diseases. *Nature* 539, 217–226. <https://doi.org/10.1038/nature20415>.
- Meyer-Luehmann, M., Spiess-Jones, T.L., Prada, C., Garcia-Alloza, M., de Calignon, A., Rozkalne, A., Koenigsnecht-Talboo, J., Holtzman, D.M., Bacskai, B.J., and Hyman, B.T. (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature* 451, 720–724. <https://doi.org/10.1038/nature06616>.
- Busche, M.A., Eichhoff, G., Adelsberger, H., Abramowski, D., Wiederhold, K.H., Haass, C., Staufenbiel, M., Konnerth, A., and Garaschuk, O. (2008). Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science* 321, 1686–1689. <https://doi.org/10.1126/science.1162844>.
- Yuan, P., Zhang, M., Tong, L., Morse, T.M., McDougal, R.A., Ding, H., Chan, D., Cai, Y., and Grutzendler, J. (2022). PLD3 affects axonal spheroids and network defects in Alzheimer's disease. *Nature* 612, 328–337. <https://doi.org/10.1038/s41586-022-05491-6>.
- Viola, K.L., and Klein, W.L. (2015). Amyloid beta oligomers in Alzheimer's disease pathogenesis, treatment, and diagnosis. *Acta Neuropathol.* 129, 183–206. <https://doi.org/10.1007/s00401-015-1386-3>.
- Tzioras, M., McGeachan, R.I., Durrant, C.S., and Spiess-Jones, T.L. (2023). Synaptic degeneration in Alzheimer disease. *Nat. Rev. Neurol.* 19, 19–38. <https://doi.org/10.1038/s41582-022-00749-z>.
- Greenberg, S.M., Bacskai, B.J., Hernandez-Guillamon, M., Pruzin, J., Sperling, R., and van Veluw, S.J. (2020). Cerebral amyloid angiopathy and Alzheimer disease - one peptide, two pathways. *Nat. Rev. Neurol.* 16, 30–42. <https://doi.org/10.1038/s41582-019-0281-2>.
- Brenowitz, W.D., Nelson, P.T., Besser, L.M., Heller, K.B., and Kukull, W.A. (2015). Cerebral amyloid angiopathy and its co-occurrence with Alzheimer's disease and other cerebrovascular neuropathologic changes. *Neurobiol. Aging* 36, 2702–2708. <https://doi.org/10.1016/j.neurobiolaging.2015.06.028>.
- Jäkel, L., De Kort, A.M., Klijn, C.J.M., Schreuder, F.H.B.M., and Verbeek, M.M. (2022). Prevalence of cerebral amyloid angiopathy: a systematic review and meta-analysis. *Alzheimers. Dement.* 18, 10–28. <https://doi.org/10.1002/alz.12366>.
- McKay, N.S., Gordon, B.A., Hornbeck, R.C., Dincer, A., Flores, S., Keefe, S.J., Joseph-Mathurin, N., Jack, C.R., Koeppe, R., Millar, P.R., et al. (2023). Positron emission tomography and magnetic resonance imaging methods and datasets within the Dominantly Inherited Alzheimer Network (DIAN). *Nat. Neurosci.* 26, 1449–1460. <https://doi.org/10.1038/s41593-023-01359-8>.
- Zetterberg, H., and Blennow, K. (2013). Biomarker evidence for uncoupling of amyloid build-up and toxicity in Alzheimer's disease. *Alzheimers. Dement.* 9, 459–462. <https://doi.org/10.1016/j.jalz.2012.07.002>.
- Karran, E., Mercken, M., and De Strooper, B. (2011). The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Rev. Drug Discov.* 10, 698–712. <https://doi.org/10.1038/nrd3505>.
- Rother, C., Uhlmann, R.E., Müller, S.A., Schelle, J., Skodras, A., Obermüller, U., Häslner, L.M., Lambert, M., Baumann, F., Xu, Y., et al. (2022). Experimental evidence for temporal uncoupling of brain A $\beta$  deposition and neurodegenerative sequelae. *Nat. Commun.* 13, 7333. <https://doi.org/10.1038/s41467-022-34538-5>.
- Long, J.M., and Holtzman, D.M. (2019). Alzheimer disease: an update on pathobiology and treatment strategies. *Cell* 179, 312–339. <https://doi.org/10.1016/j.cell.2019.09.001>.
- Karran, E., and De Strooper, B. (2022). The amyloid hypothesis in Alzheimer disease: new insights from new therapeutics. *Nat. Rev. Drug Discov.* 21, 306–318. <https://doi.org/10.1038/s41573-022-00391-w>.
- DeTure, M.A., and Dickson, D.W. (2019). The neuropathological diagnosis of Alzheimer's disease. *Mol. Neurodegener.* 14, 32. <https://doi.org/10.1186/s13024-019-0333-5>.
- Ji, C., and Sigurdsson, E.M. (2021). Current status of clinical trials on tau immunotherapies. *Drugs* 81, 1135–1152. <https://doi.org/10.1007/s40265-021-01546-6>.

31. Imbimbo, B.P., Balducci, C., Ippati, S., and Watling, M. (2023). Initial failures of anti-tau antibodies in Alzheimer's disease are reminiscent of the amyloid-beta story. *Neural Regen. Res.* *18*, 117–118. <https://doi.org/10.4103/1673-5374.340409>.
32. Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., et al. (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* *400*, 173–177. <https://doi.org/10.1038/22124>.
33. Brody, D.L., and Holtzman, D.M. (2008). Active and passive immunotherapy for neurodegenerative disorders. *Annu. Rev. Neurosci.* *31*, 175–193. <https://doi.org/10.1146/annurev.neuro.31.060407.125529>.
34. Orgogozo, J.M., Gilman, S., Dartigues, J.F., Laurent, B., Puel, M., Kirby, L.C., Jouanny, P., Dubois, B., Eisner, L., Flitman, S., et al. (2003). Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* *61*, 46–54. <https://doi.org/10.1212/01.wnl.0000073623.84147.a8>.
35. Hock, C., Konietzko, U., Streffer, J.R., Tracy, J., Signorelli, A., Müller-Tillmanns, B., Lemke, U., Henke, K., Moritz, E., Garcia, E., et al. (2003). Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. *Neuron* *38*, 547–554. [https://doi.org/10.1016/s0896-6273\(03\)00294-0](https://doi.org/10.1016/s0896-6273(03)00294-0).
36. Nicoll, J.A., Barton, E., Boche, D., Neal, J.W., Ferrer, I., Thompson, P., Vlachouli, C., Wilkinson, D., Bayer, A., Games, D., et al. (2006). Abeta species removal after abeta42 immunization. *J. Neuropathol. Exp. Neurol.* *65*, 1040–1048. <https://doi.org/10.1097/01.jnen.0000240466.10758.ce>.
37. Nicoll, J.A.R., Buckland, G.R., Harrison, C.H., Page, A., Harris, S., Love, S., Neal, J.W., Holmes, C., and Boche, D. (2019). Persistent neuropathological effects 14 years following amyloid-beta immunization in Alzheimer's disease. *Brain* *142*, 2113–2126. <https://doi.org/10.1093/brain/awz142>.
38. Villain, N., Planche, V., and Levy, R. (2022). High-clearance anti-amyloid immunotherapies in Alzheimer's disease. Part 1: meta-analysis and review of efficacy and safety data, and medico-economical aspects. *Rev. Neurol. (Paris)* *178*, 1011–1030. <https://doi.org/10.1016/j.neurol.2022.06.012>.
39. La Joie, R., Ayakta, N., Seeley, W.W., Borys, E., Boxer, A.L., DeCarli, C., Dore, V., Grinberg, L.T., Huang, E., Hwang, J.H., et al. (2019). Multisite study of the relationships between antemortem [<sup>11</sup>C]PIB-PET Centiloid values and postmortem measures of Alzheimer's disease neuropathology. *Alzheimers Dement.* *15*, 205–216. <https://doi.org/10.1016/j.jalz.2018.09.001>.
40. Fritschi, S.K., Langer, F., Kaeser, S.A., Maia, L.F., Portelius, E., Pinotsi, D., Kaminski, C.F., Winkler, D.T., Maetzler, W., Keyvani, K., et al. (2014). Highly potent soluble amyloid-beta seeds in human Alzheimer brain but not cerebrospinal fluid. *Brain* *137*, 2909–2915. <https://doi.org/10.1093/brain/awu255>.
41. Ye, L., Rasmussen, J., Kaeser, S.A., Marzesco, A.M., Obermüller, U., Mahler, J., Schelle, J., Odenthal, J., Krüger, C., Fritschi, S.K., et al. (2017). Abeta seeding potency peaks in the early stages of cerebral beta-amyloidosis. *EMBO Rep.* *18*, 1536–1544. <https://doi.org/10.15252/embr.201744067>.
42. Yang, Y., Arseni, D., Zhang, W., Huang, M., Lövestam, S., Schweighauser, M., Kotecha, A., Murzin, A.G., Peak-Chew, S.Y., Macdonald, J., et al. (2022). Cryo-EM structures of amyloid-beta 42 filaments from human brains. *Science* *375*, 167–172. <https://doi.org/10.1126/science.abm7285>.
43. Plotkin, S.S., and Cashman, N.R. (2020). Passive immunotherapies targeting Abeta and tau in Alzheimer's disease. *Neurobiol. Dis.* *144*, 105010. <https://doi.org/10.1016/j.nbd.2020.105010>.
44. Uhlmann, R.E., Rother, C., Rasmussen, J., Schelle, J., Bergmann, C., Ullrich Gavilanes, E.M., Fritschi, S.K., Buehler, A., Baumann, F., Skodras, A., et al. (2020). Acute targeting of pre-amyloid seeds in transgenic mice reduces Alzheimer-like pathology later in life. *Nat. Neurosci.* *23*, 1580–1588. <https://doi.org/10.1038/s41593-020-00737-w>.
45. Salloway, S., Farlow, M., McDade, E., Clifford, D.B., Wang, G., Llibre-Guerra, J.J., Hitchcock, J.M., Mills, S.L., Santacruz, A.M., Aschenbrenner, A.J., et al. (2021). A trial of gantenerumab or solanezumab in dominantly inherited Alzheimer's disease. *Nat. Med.* *27*, 1187–1196. <https://doi.org/10.1038/s41591-021-01369-8>.
46. Pontecorvo, M.J., Lu, M., Burnham, S.C., Schade, A.E., Dage, J.L., Shcherbinin, S., Collins, E.C., Sims, J.R., and Mintun, M.A. (2022). Association of donanemab treatment with exploratory plasma biomarkers in early symptomatic Alzheimer disease: a secondary analysis of the TRAILBLAZER-ALZ randomized clinical trial. *JAMA Neurol.* *79*, 1250–1259. <https://doi.org/10.1001/jamaneurol.2022.3392>.
47. U.S. Food & Drug Administration Peripheral and Central Nervous System Drugs Advisory Committee (2020). Aducanumab for the TREATMENT of Alzheimer's disease. <https://www.fda.gov/media/143577/download>.
48. Mintun, M.A., Lo, A.C., Duggan Evans, C., Wessels, A.M., Ardayfio, P.A., Andersen, S.W., Shcherbinin, S., Sparks, J., Sims, J.R., Brys, M., et al. (2021). Donanemab in early Alzheimer's disease. *N. Engl. J. Med.* *384*, 1691–1704. <https://doi.org/10.1056/NEJMoa2100708>.
49. Bacioglu, M., Maia, L.F., Preische, O., Schelle, J., Apel, A., Kaeser, S.A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., et al. (2016). Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. *Neuron* *91*, 56–66. <https://doi.org/10.1016/j.neuron.2016.05.018>.
50. Gafson, A.R., Barthélemy, N.R., Bomont, P., Carare, R.O., Durham, H.D., Julien, J.P., Kuhle, J., Leppert, D., Nixon, R.A., Weller, R.O., et al. (2020). Neurofilaments: neurobiological foundations for biomarker applications. *Brain* *143*, 1975–1998. <https://doi.org/10.1093/brain/awaa098>.
51. Preische, O., Schultz, S.A., Apel, A., Kuhle, J., Kaeser, S.A., Barro, C., Gräber, S., Kuder-Buletta, E., LaFougere, C., Laske, C., et al. (2019). Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat. Med.* *25*, 277–283. <https://doi.org/10.1038/s41591-018-0304-3>.
52. Alves, F., Kalinowski, P., and Ayton, S. (2023). Accelerated brain volume loss caused by anti-beta-amyloid drugs: a systematic review and meta-analysis. *Neurology* *100*, e2114–e2124. <https://doi.org/10.1212/WNL.0000000000207156>.
53. Barkhof, F., and Knopman, D.S. (2023). Brain shrinkage in anti-beta-amyloid Alzheimer trials: neurodegeneration or pseudoatrophy? *Neurology* *100*, 941–942. <https://doi.org/10.1212/WNL.0000000000207268>.
54. Holdridge, K.C., Yaari, R., Hoban, D.B., Andersen, S., and Sims, J.R. (2023). Targeting amyloid beta in Alzheimer's disease: meta-analysis of low-dose solanezumab in Alzheimer's disease with mild dementia studies. *Alzheimers Dement.* <https://doi.org/10.1002/alz.13031>.
55. AlzForum (2023). Legacy of A4 secondary prevention study goes beyond negative result. <https://www.alzforum.org/news/research-news/legacy-a4-secondary-prevention-study-goes-beyond-negative-result>.
56. Herzig, M.C., Van Nostrand, W.E., and Jucker, M. (2006). Mechanism of cerebral beta-amyloid angiopathy: murine and cellular models. *Brain Pathol.* *16*, 40–54. <https://doi.org/10.1111/j.1750-3639.2006.tb00560.x>.
57. Kollmer, M., Close, W., Funk, L., Rasmussen, J., Bsoul, A., Schierhorn, A., Schmidt, M., Sigurdson, C.J., Jucker, M., and Fändrich, M. (2019). Cryo-EM structure and polymorphism of Abeta amyloid fibrils purified from Alzheimer's brain tissue. *Nat. Commun.* *10*, 4760. <https://doi.org/10.1038/s41467-019-12683-8>.
58. Lannfelt, L., Moller, C., Basun, H., Osswald, G., Sehlin, D., Satlin, A., Logovinsky, V., and Gellerfors, P. (2014). Perspectives on future Alzheimer therapies: amyloid-beta protofibrils – a new target for immunotherapy with BAN2401 in Alzheimer's disease. *Alzheimers Res. Ther.* *6*, 16. <https://doi.org/10.1186/alzrt246>.
59. Nilsberth, C., Westlind-Danielsson, A., Eckman, C.B., Condron, M.M., Axelman, K., Forsell, C., Sten, C., Luthman, J., Teplow, D.B., Younkin, S.G., et al. (2001). The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nat. Neurosci.* *4*, 887–893. <https://doi.org/10.1038/nn0901-887>.



60. Yang, Y., Zhang, W., Murzin, A.G., Schweighauser, M., Huang, M., Lövestam, S., Peak-Chew, S.Y., Saito, T., Saido, T.C., Macdonald, J., et al. (2023). Cryo-EM structures of amyloid-beta filaments with the Arctic mutation (E22G) from human and mouse brains. *Acta Neuropathol.* *145*, 325–333. <https://doi.org/10.1007/s00401-022-02533-1>.
61. Linse, S., Scheidt, T., Bernfur, K., Vendruscolo, M., Dobson, C.M., Cohen, S.I.A., Sileikis, E., Lundqvist, M., Qian, F., O'Malley, T., et al. (2020). Kinetic fingerprints differentiate the mechanisms of action of anti-Abeta antibodies. *Nat. Struct. Mol. Biol.* *27*, 1125–1133. <https://doi.org/10.1038/s41594-020-0505-6>.
62. Frost, J.L., Le, K.X., Cynis, H., Ekpo, E., Kleinschmidt, M., Palmour, R.M., Ervin, F.R., Snigdha, S., Cotman, C.W., Saido, T.C., et al. (2013). Pyroglutamate-3 amyloid-beta deposition in the brains of humans, non-human primates, canines, and Alzheimer disease-like transgenic mouse models. *Am. J. Pathol.* *183*, 369–381. <https://doi.org/10.1016/j.ajpath.2013.05.005>.
63. Rijal Upadhaya, A., Kosterin, I., Kumar, S., von Arnim, C.A., Yamaguchi, H., Fändrich, M., Walter, J., and Thal, D.R. (2014). Biochemical stages of amyloid-beta peptide aggregation and accumulation in the human brain and their association with symptomatic and pathologically preclinical Alzheimer's disease. *Brain* *137*, 887–903. <https://doi.org/10.1093/brain/awt362>.
64. Li, X., Ospitalieri, S., Robberechts, T., Hofmann, L., Schmid, C., Rijal Upadhaya, A., Koper, M.J., von Arnim, C.A.F., Kumar, S., Willem, M., et al. (2022). Seeding, maturation and propagation of amyloid beta-peptide aggregates in Alzheimer's disease. *Brain* *145*, 3558–3570. <https://doi.org/10.1093/brain/awac202>.
65. Scarce-Levie, K., Sanchez, P.E., and Lewcock, J.W. (2020). Leveraging preclinical models for the development of Alzheimer disease therapeutics. *Nat. Rev. Drug Discov.* *19*, 447–462. <https://doi.org/10.1038/s41573-020-0065-9>.
66. Lowe, S.L., Willis, B.A., Hawdon, A., Natanegara, F., Chua, L., Foster, J., Shcherbinin, S., Ardayio, P., and Sims, J.R. (2021). Donanemab (LY3002813) dose-escalation study in Alzheimer's disease. *Alzheimers. Dement. (N Y)* *7*, e12112. <https://doi.org/10.1002/trc2.12112>.
67. McDade, E., and Bateman, R.J. (2017). Stop Alzheimer's before it starts. *Nature* *547*, 153–155. <https://doi.org/10.1038/547153a>.
68. Karlinski, R.A., Rosenthal, A., Kobayashi, D., Pons, J., Alamed, J., Mercer, M., Li, Q., Gordon, M.N., Gottschall, P.E., and Morgan, D. (2009). Suppression of amyloid deposition leads to long-term reductions in Alzheimer's pathologies in Tg2576 mice. *J. Neurosci.* *29*, 4964–4971. <https://doi.org/10.1523/JNEUROSCI.4560-08.2009>.
69. Niewoehner, J., Bohrmann, B., Collin, L., Urich, E., Sade, H., Maier, P., Rueger, P., Stracke, J.O., Lau, W., Tissot, A.C., et al. (2014). Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron* *81*, 49–60. <https://doi.org/10.1016/j.neuron.2013.10.061>.
70. Bathini, P., Sun, T., Schenk, M., Schilling, S., McDannold, N.J., and Lemere, C.A. (2022). Acute effects of focused ultrasound-induced blood-brain barrier opening on anti-Pyroglu3 Abeta antibody delivery and immune responses. *Biomolecules* *12*. <https://doi.org/10.3390/biom12070951>.
71. Hansson, O. (2021). Biomarkers for neurodegenerative diseases. *Nat. Med.* *27*, 954–963. <https://doi.org/10.1038/s41591-021-01382-x>.
72. Teunissen, C.E., Verberk, I.M.W., Thijssen, E.H., Vermunt, L., Hansson, O., Zetterberg, H., van der Flier, W.M., Mielke, M.M., and Del Campo, M. (2022). Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. *Lancet Neurol.* *21*, 66–77. [https://doi.org/10.1016/S1474-4422\(21\)00361-6](https://doi.org/10.1016/S1474-4422(21)00361-6).
73. Ikonomic, M.D., Abrahamson, E.E., Price, J.C., Hamilton, R.L., Mathis, C.A., Paljug, W.R., Debnath, M.L., Cohen, A.D., Mizukami, K., DeKosky, S.T., et al. (2012). Early AD pathology in a [C-11]PiB-negative case: a PiB-amyloid imaging, biochemical, and immunohistochemical study. *Acta Neuropathol.* *123*, 433–447. <https://doi.org/10.1007/s00401-012-0943-2>.
74. Pletnikova, O., Rudow, G.L., Hyde, T.M., Kleinman, J.E., Ali, S.Z., Bharadwaj, R., Gangadeen, S., Crain, B.J., Fowler, D.R., Rubio, A.I., et al. (2015). Alzheimer lesions in the autopsied brains of people 30 to 50 years of age. *Cogn. Behav. Neurol.* *28*, 144–152. <https://doi.org/10.1097/WNN.000000000000071>.
75. Jagust, W.J., and Landau, S.M.; Alzheimer's Disease Neuroimaging Initiative (2021). Temporal dynamics of beta-amyloid accumulation in aging and Alzheimer disease. *Neurology* *96*, e1347–e1357. <https://doi.org/10.1212/WNL.00000000000011524>.
76. Barthélemy, N.R., Li, Y., Joseph-Mathurin, N., Gordon, B.A., Hassenstab, J., Benzinger, T.L.S., Buckles, V., Fagan, A.M., Perrin, R.J., Goate, A.M., et al. (2020). A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat. Med.* *26*, 398–407. <https://doi.org/10.1038/s41591-020-0781-z>.
77. Ashton, N.J., Janelidze, S., Mattsson-Carlsson, N., Binette, A.P., Strandberg, O., Brum, W.S., Karikari, T.K., González-Ortiz, F., Di Molfetta, G., Meda, F.J., et al. (2022). Differential roles of Aβ42/40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring. *Nat. Med.* *28*, 2555–2562. <https://doi.org/10.1038/s41591-022-02074-w>.
78. Pereira, J.B., Janelidze, S., Smith, R., Mattsson-Carlsson, N., Palmqvist, S., Teunissen, C.E., Zetterberg, H., Stomrud, E., Ashton, N.J., Blennow, K., et al. (2021). Plasma GFAP is an early marker of amyloid-beta but not tau pathology in Alzheimer's disease. *Brain* *144*, 3505–3516. <https://doi.org/10.1093/brain/awab223>.
79. Morenas-Rodríguez, E., Li, Y., Nuscher, B., Franzmeier, N., Xiong, C., Suárez-Calvet, M., Fagan, A.M., Schultz, S., Gordon, B.A., Benzinger, T.L.S., et al. (2022). Soluble TREM2 in CSF and its association with other biomarkers and cognition in autosomal-dominant Alzheimer's disease: a longitudinal observational study. *Lancet Neurol.* *21*, 329–341. [https://doi.org/10.1016/S1474-4422\(22\)00027-8](https://doi.org/10.1016/S1474-4422(22)00027-8).
80. Kaeser, S.A., Häslér, L.M., Lambert, M., Bergmann, C., Böttelbergs, A., Theunis, C., Mercken, M., and Jucker, M. (2022). CSF p-tau increase in response to Aβeta-type and Danish-type cerebral amyloidosis and in the absence of neurofibrillary tangles. *Acta Neuropathol.* *143*, 287–290. <https://doi.org/10.1007/s00401-021-02400-5>.
81. Eninger, T., Müller, S.A., Bacioglu, M., Schweighauser, M., Lambert, M., Maia, L.F., Neher, J.J., Hornfeck, S.M., Obermüller, U., Kleinberger, G., et al. (2022). Signatures of glial activity can be detected in the CSF proteome. *Proc. Natl. Acad. Sci. USA* *119*. e2119804119. <https://doi.org/10.1073/pnas.2119804119>.
82. Mok, T.H., Nihat, A., Majbour, N., Sequeira, D., Holm-Mercer, L., Coysh, T., Darwent, L., Batchelor, M., Groveman, B.R., Orr, C.D., et al. (2023). Seed amplification and neurodegeneration marker trajectories in individuals at risk of prion disease. *Brain* *146*, 2570–2583. <https://doi.org/10.1093/brain/awad101>.
83. Siderowf, A., Concha-Marambio, L., Lafontant, D.E., Farris, C.M., Ma, Y., Urenia, P.A., Nguyen, H., Alcalay, R.N., Chahine, L.M., Foroud, T., et al. (2023). Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using alpha-synuclein seed amplification: a cross-sectional study. *Lancet Neurol.* *22*, 407–417. [https://doi.org/10.1016/S1474-4422\(23\)00109-6](https://doi.org/10.1016/S1474-4422(23)00109-6).
84. Okuzumi, A., Hatano, T., Matsumoto, G., Nojiri, S., Ueno, S.I., Imamichi-Tatano, Y., Kimura, H., Kakuta, S., Kondo, A., Fukuhara, T., et al. (2023). Propagative alpha-synuclein seeds as serum biomarkers for synucleinopathies. *Nat. Med.* *29*, 1448–1455. <https://doi.org/10.1038/s41591-023-02358-9>.
85. Salloway, S., Chalkias, S., Barkhof, F., Burkett, P., Barakos, J., Purcell, D., Suhy, J., Forrestal, F., Tian, Y., Umans, K., et al. (2022). Amyloid-related imaging abnormalities in 2 phase 3 studies evaluating aducanumab in patients with early Alzheimer disease. *JAMA Neurol.* *79*, 13–21. <https://doi.org/10.1001/jamaneurol.2021.4161>.

86. Roytman, M., Mashriqi, F., Al-Tawil, K., Schulz, P.E., Zaharchuk, G., Benzinger, T.L.S., and Franceschi, A.M. (2023). Amyloid-related imaging abnormalities: an update. *AJR Am. J. Roentgenol.* 220, 562–574. <https://doi.org/10.2214/AJR.22.28461>.
87. Sperling, R., Salloway, S., Brooks, D.J., Tampieri, D., Barakos, J., Fox, N.C., Raskind, M., Sabbagh, M., Honig, L.S., Porsteinsson, A.P., et al. (2012). Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis. *Lancet Neurol.* 11, 241–249. [https://doi.org/10.1016/S1474-4422\(12\)70015-7](https://doi.org/10.1016/S1474-4422(12)70015-7).
88. Reish, N.J., Jamshidi, P., Stamm, B., Flanagan, M.E., Sugg, E., Tang, M., Donohue, K.L., McCord, M., Krumpelman, C., Mesulam, M.M., et al. (2023). Multiple cerebral hemorrhages in a patient receiving lecanemab and treated with t-PA for stroke. *N. Engl. J. Med.* 388, 478–479. <https://doi.org/10.1056/NEJMc2215148>.
89. Solopova, E., Romero-Fernandez, W., Harmsen, H., Ventura-Antunes, L., Wang, E., Shostak, A., Maldonado, J., Donahue, M., Schultz, D., Coyne, T.M., et al. (2023). Fatal iatrogenic cerebral amyloid-related encephalitis in a patient treated with lecanemab for Alzheimer's disease: neuroimaging and neuropathology. Preprint at medRxiv <https://doi.org/10.1101/2023.04.26.23289061>.
90. Sveikata, L., Charidimou, A., and Viswanathan, A. (2022). Vessels sing their ARIAs: the role of vascular amyloid in the age of aducanumab. *Stroke* 53, 298–302. <https://doi.org/10.1161/STROKEAHA.121.036873>.
91. Pfeifer, M., Boncristiano, S., Bondolfi, L., Stalder, A., Deller, T., Staufenbiel, M., Mathews, P.M., and Jucker, M. (2002). Cerebral hemorrhage after passive anti-Aβ immunotherapy. *Science* 298, 1379. <https://doi.org/10.1126/science.1078259>.
92. Plowey, E.D., Bussiere, T., Rajagovindan, R., Sebalusky, J., Hamann, S., von Hehn, C., Castrillo-Viguera, C., Sandrock, A., Budd Haerberlein, S., van Dyck, C.H., et al. (2022). Alzheimer disease neuropathology in a patient previously treated with aducanumab. *Acta Neuropathol.* 144, 143–153. <https://doi.org/10.1007/s00401-022-02433-4>.
93. Scherlek, A.A., Kozberg, M.G., Nicoll, J.A.R., Perosa, V., Freeze, W.M., van der Weerd, L., Bacskai, B.J., Greenberg, S.M., Frosch, M.P., Boche, D., et al. (2022). Histopathological correlates of haemorrhagic lesions on ex vivo magnetic resonance imaging in immunized Alzheimer's disease cases. *Brain Commun.* 4, fcac021. <https://doi.org/10.1093/brain-comms/fcac021>.
94. Boche, D., Zotova, E., Weller, R.O., Love, S., Neal, J.W., Pickering, R.M., Wilkinson, D., Holmes, C., and Nicoll, J.A. (2008). Consequence of Aβ immunization on the vasculature of human Alzheimer's disease brain. *Brain* 131, 3299–3310. <https://doi.org/10.1093/brain/awn261>.
95. Sakai, K., Boche, D., Carare, R., Johnston, D., Holmes, C., Love, S., and Nicoll, J.A. (2014). Aβ immunotherapy for Alzheimer's disease: effects on apoE and cerebral vasculopathy. *Acta Neuropathol.* 128, 777–789. <https://doi.org/10.1007/s00401-014-1340-9>.
96. Winkler, D.T., Bondolfi, L., Herzig, M.C., Jann, L., Calhoun, M.E., Wiederhold, K.H., Tolnay, M., Staufenbiel, M., and Jucker, M. (2001). Spontaneous hemorrhagic stroke in a mouse model of cerebral amyloid angiopathy. *J. Neurosci.* 21, 1619–1627. <https://doi.org/10.1523/JNEUROSCI.21-05-01619.2001>.
97. Heffernan, A.L., Chidgey, C., Peng, P., Masters, C.L., and Roberts, B.R. (2016). The neurobiology and age-related prevalence of the epsilon4 allele of Apolipoprotein E in Alzheimer's disease cohorts. *J. Mol. Neurosci.* 60, 316–324. <https://doi.org/10.1007/s12031-016-0804-x>.
98. Nelson, P.T., Pious, N.M., Jicha, G.A., Wilcock, D.M., Fardo, D.W., Estus, S., and Rebeck, G.W. (2013). APOE-epsilon2 and APOE-epsilon4 correlate with increased amyloid accumulation in cerebral vasculature. *J. Neuropathol. Exp. Neurol.* 72, 708–715. <https://doi.org/10.1097/NEN.0b013e31829a25b9>.
99. Foley, K.E., and Wilcock, D.M. (2022). Vascular considerations for amyloid immunotherapy. *Curr. Neurol. Neurosci. Rep.* 22, 709–719. <https://doi.org/10.1007/s11910-022-01235-1>.
100. Charidimou, A., Boulouis, G., Frosch, M.P., Baron, J.C., Pasi, M., Albu-Cher, J.F., Banerjee, G., Barbato, C., Bonneville, F., Brandner, S., et al. (2022). The Boston criteria version 2.0 for cerebral amyloid angiopathy: a multicentre, retrospective, MRI-neuropathology diagnostic accuracy study. *Lancet Neurol.* 21, 714–725. [https://doi.org/10.1016/S1474-4422\(22\)00208-3](https://doi.org/10.1016/S1474-4422(22)00208-3).
101. Wagner, J., Degenhardt, K., Veit, M., Louros, N., Konstantoulea, K., Skodras, A., Wild, K., Liu, P., Obermüller, U., Bansal, V., et al. (2022). Medin co-aggregates with vascular amyloid-beta in Alzheimer's disease. *Nature* 612, 123–131. <https://doi.org/10.1038/s41586-022-05440-3>.
102. Rasmussen, M.K., Mestre, H., and Nedergaard, M. (2018). The glymphatic pathway in neurological disorders. *Lancet Neurol.* 17, 1016–1024. [https://doi.org/10.1016/S1474-4422\(18\)30318-1](https://doi.org/10.1016/S1474-4422(18)30318-1).
103. Agarwal, N., and Carare, R.O. (2020). Cerebral vessels: an overview of anatomy, physiology, and role in the drainage of fluids and solutes. *Front. Neurol.* 11, 611485. <https://doi.org/10.3389/fneur.2020.611485>.
104. Drieu, A., Du, S., Storck, S.E., Rustenhoven, J., Papadopoulos, Z., Dykstra, T., Zhong, F., Kim, K., Blackburn, S., Mamuladze, T., et al. (2022). Parenchymal border macrophages regulate the flow dynamics of the cerebrospinal fluid. *Nature* 611, 585–593. <https://doi.org/10.1038/s41586-022-05397-3>.
105. Rustenhoven, J., and Kipnis, J. (2022). Brain borders at the central stage of neuroimmunology. *Nature* 612, 417–429. <https://doi.org/10.1038/s41586-022-05474-7>.
106. Wilson, D.M., 3rd, Cookson, M.R., Van Den Bosch, L., Zetterberg, H., Holtzman, D.M., and Dewachter, I. (2023). Hallmarks of neurodegenerative diseases. *Cell* 186, 693–714. <https://doi.org/10.1016/j.cell.2022.12.032>.
107. Andersson, E., Schultz, N., Saito, T., Saido, T.C., Blennow, K., Gouras, G.K., Zetterberg, H., and Hansson, O. (2023). Cerebral Aβ deposition precedes reduced cerebrospinal fluid and serum Aβ42/Aβ40 ratios in the App<sup>NL-F/NL-F</sup> knock-in mouse model of Alzheimer's disease. *Alzheimers Res. Ther.* 15, 64. <https://doi.org/10.1186/s13195-023-01196-8>.
108. Xiong, M., Jiang, H., Serrano, J.R., Gonzales, E.R., Wang, C., Gratzue, M., Hoyle, R., Bien-Ly, N., Silverman, A.P., Sullivan, P.M., et al. (2021). APOE immunotherapy reduces cerebral amyloid angiopathy and amyloid plaques while improving cerebrovascular function. *Sci. Transl. Med.* 13. <https://doi.org/10.1126/scitranslmed.abd7522>.
109. Zhao, Y., Zheng, Q., Hong, Y., Gao, Y., Hu, J., Lang, M., Zhang, H., Zhou, Y., Luo, H., Zhang, X., et al. (2023). β2-Microglobulin coaggregates with Aβ and contributes to amyloid pathology and cognitive deficits in Alzheimer's disease model mice. *Nat. Neurosci.* 26, 1170–1184. <https://doi.org/10.1038/s41593-023-01352-1>.
110. Schlepckow, K., Monroe, K.M., Kleinberger, G., Cantuti-Castelvetri, L., Parhizkar, S., Xia, D., Willem, M., Werner, G., Pettkus, N., Brunner, B., et al. (2020). Enhancing protective microglial activities with a dual function TREM2 antibody to the stalk region. *EMBO Mol. Med.* 12, e11227. <https://doi.org/10.15252/emmm.201911227>.
111. Zhao, P., Xu, Y., Jiang, L., Fan, X., Li, L., Li, X., Arase, H., Zhao, Y., Cao, W., Zheng, H., et al. (2022). A tetravalent TREM2 agonistic antibody reduced amyloid pathology in a mouse model of Alzheimer's disease. *Sci. Transl. Med.* 14, eabq0095. <https://doi.org/10.1126/scitranslmed.abq0095>.
112. Wisniewski, T., and Goñi, F. (2015). Immunotherapeutic approaches for Alzheimer's disease. *Neuron* 85, 1162–1176. <https://doi.org/10.1016/j.neuron.2014.12.064>.
113. Schenk, D. (2002). Amyloid-beta immunotherapy for Alzheimer's disease: the end of the beginning. *Nat. Rev. Neurosci.* 3, 824–828. <https://doi.org/10.1038/nrn938>.
114. Winblad, B., Graf, A., Riviere, M.E., Andreasen, N., and Ryan, J.M. (2014). Active immunotherapy options for Alzheimer's disease. *Alzheimers Res. Ther.* 6, 7. <https://doi.org/10.1186/alzrt237>.

115. Vogt, A.S., Jennings, G.T., Mohsen, M.O., Vogel, M., and Bachmann, M.F. (2023). Alzheimer's disease: a brief history of immunotherapies targeting amyloid  $\beta$ . *Int. J. Mol. Sci.* 24. <https://doi.org/10.3390/ijms24043895>.
116. Boyle, P.A., Wang, T., Yu, L., Wilson, R.S., Dawe, R., Arfanakis, K., Schneider, J.A., and Bennett, D.A. (2021). To what degree is late life cognitive decline driven by age-related neuropathologies? *Brain* 144, 2166–2175. <https://doi.org/10.1093/brain/awab092>.
117. Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C.E., Cummings, J., and van der Flier, W.M. (2021). Alzheimer's disease. *Lancet* 397, 1577–1590. [https://doi.org/10.1016/S0140-6736\(20\)32205-4](https://doi.org/10.1016/S0140-6736(20)32205-4).