The Journal of Prevention of Alzheimer's Disease 13 (2026) 100669

Contents lists available at ScienceDirect

The Journal of Prevention of Alzheimer's Disease

journal homepage: www.elsevier.com/locate/tjpad

Original Article

Autopsy findings in Alzheimer's disease clinical trial participants demonstrate a high frequency of off-target coexisting pathologic features

E. Pinar Coskun [a,b,*], Justin Barber [b], Lauren Bojarski [a], Christopher J. McLouth [a,c], Erin L. Abner [b,d], Linda J. Van Eldik [b,e], Peter T. Nelson [b,f], Gregory A. Jicha [a,b]

[a] Department of Neurology, College of Medicine, University of Kentucky, Lexington, KY, United States
[b] Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, United States
[c] Department of of Biostatistics, College of Public Health, University of Kentucky, Lexington, KY, United States
[d] Department of Epidemiology and Environmental Health, College of Public Health, University of Kentucky, Lexington, KY, United States
[e] Department of Neuroscience, College of Medicine, University of Kentucky, Lexington, KY, United States
[f] Department of Pathology & Laboratory Medicine, College of Medicine, University of Kentucky, Lexington, KY, United States

ARTICLE INFO

Keywords:
Alzheimer disease / pathology
Comorbidity
Dementia / etiology
Clinical trials as topic / methods
Biomarkers / diagnostic use

ABSTRACT

Background: Having multiple comorbid neuropathologic features may confound the results of interventional trials that were designed to target a specific pathophysiologic mechanism in Alzheimer's disease (AD) and or related dementias (ADRD). However, it is unknown what percentage of individuals undergoing AD/ADRD interventional studies have mixed pathologies.

Objective: To characterize the spectrum of coexisting neuropathologies in brains of AD/ADRD clinical trial participants to inform trial design and therapeutic strategies

Methods: Autopsied participants from the University of Kentucky Alzheimer Disease Research Center (UK-ADRC) community-based cohort who died between January 2005, and February 2024 were included and queried retrospectively for participation in therapeutic interventional trials. Of a total of 614 autopsied cases, 67had been enrolled in one of the following types of clinical trials: cognitively normal participants in prevention trials for AD/ADRD (designated group **P**; n = 21); interventions for mild cognitive impairment or early dementia (**MCI/D**; n = 26); and, interventions for vascular cognitive impairment (**V**; n = 20). The trial-engaged groups were compared to the trial-naïve group in terms of their demographic, clinical, and genetic characteristics. Pathological features (amyloid-β, tau, α-synuclein, TDP-43, and cerebrovascular disease) were assessed using consensus-based neuropathologic methods.

Results: All interventions were designed to target only a single pathologic feature. The trial-engaged group did not differ significantly from those who were trial-naïve with respect to demographic, genetic (*APOE*), or clinical characteristics, except for a marginally higher level of education among trial participants ($p = 0.04$). Pure on-target pathology (i.e., only one isolated pathology was found at autopsy that was the signature pathology targeted by the intervention) was only seen in 10, 23, and 29 % of the engaged participants of V, MCI/D and P trials respectively. Comorbid pathologies were common in all three groups. On average, the trial-engaged groups altogether had a mean of 2.54 pathologic features/person, whereas the MCI/D trial-engaged group had a mean of 3.2 pathologic features/person.

Conclusion: Multi-etiology dementia was the norm rather than the exception for AD/ADRD trial participants. Recognizing the heterogeneity of multiple pathologies in clinical trial participants may enable the development of improved inclusion/exclusion criteria, as well as the rational use of antemortem biomarkers to stratify the likelihood of mixed comorbid pathologies that may be undesirable for single-target interventional studies. Further, multitargeted treatment strategies may be required in future trials of disease-modifying agents.

* Corresponding author at: Assistant Professor of Neurology/Geriatric Neurology, Sanders-Brown Center on Aging, University of Kentucky, Dept. Neurology, 2199 Harrodsburg Rd, Lexington, KY, 40504, United States.
E-mail address: pinar.coskun@uky.edu (E.P. Coskun).

https://doi.org/10.1016/j.tjpad.2026.100669
Received 4 September 2025; Received in revised form 17 July 2026; Accepted 5 August 2026
Available online 15 September 2026

1. Introduction

Over the past three decades, significant progress has been made in developing treatment options for Alzheimer's disease (AD)-type dementia, resulting in both symptomatic and disease-modifying drugs. Notably, two drugs that target amyloid plaques received full FDA approval in 2023 and 2024 [1–3]. These disease-modifying agents slow global cognitive decline by 22 to 40 % over 18 months, but have no documented evidence of a higher degree of clinical efficacy despite robust clearing of amyloid plaques from the brain [4–6]. The evolving landscape of AD drug development, 164 ongoing clinical trials assessing 127 unique drugs for AD treatment- including 32 drugs in Phase 3, 81 in Phase 2, and 25 in Phase 1 [3], underscores the urgency of improving therapeutic outcomes.

The large and still growing investment in research and development for drugs designed to prevent amnestic dementia highlights the need to critically evaluate current therapeutic strategies and their effectiveness. Key questions arise: Are these therapies targeting the appropriate mechanisms, or might alternative pathways offer greater efficacy and improved safety? Are the current interventions being administered too late in the disease progression to yield meaningful benefits? And importantly, are comorbid pathologies that are left unaddressed by these treatments, undermining their effectiveness despite the drugs successfully engaging their intended biological targets? Addressing these challenges is crucial for advancing the field and achieving better outcomes.

Clinical studies and epidemiologic research have revealed that mixed pathology is common in (AD-type) amnestic dementia [7–9]. The co-occurrence of multiple brain pathologies in later life, such as cerebrovascular disease (CVD), Lewy body disease (LBD), or TDP-43 proteinopathies, alongside amyloid-β and tau pathologies, is the norm rather than the exception [10]. This complexity may include even clinical trial participants who are carefully selected based on strict inclusion and exclusion criteria to minimize the effects of comorbid conditions that may also contribute to cognitive decline and dementia. If comorbid neuropathologies are prevalent in research participants enrolled in clinical trials testing the safety and efficacy of disease-modifying agents that target a single proteinopathy and/or mechanism, it is expected that the beneficial effects, should they exist, would be blunted or even undetectable despite the biological efficacy of the trial drug [11]. Investigating the prevalence of mixed pathologic features in clinical trial participants is thus an important step forward for advancing therapeutic strategies.

To date, systematic investigations of the frequency and implications of comorbid neuropathologies among ADRD trial participants have been limited to small autopsy case series drawn from single clinical trials [12–17]. To better characterize the neuropathologic substrates and frequency of multi-etiology dementia that may contribute to therapeutic failures, and to better inform future research and clinical trial design, we retrospectively analyzed neuropathologies of clinical trial volunteers who were enrolled in the community-based autopsy cohort at the University of Kentucky Alzheimer's Disease Research Center (UK-ADRC).

2. Methods

2.1. Study sample

The UK-ADRC enrolls research volunteers in a community-based longitudinal cohort that actively recruits adults aged 70 and older from the Central Kentucky region and has been described previously along with recruitment details [18,19]. Briefly, volunteers agree to be followed approximately annually for cognitive, physical, and neurological examination and to brain donation and autopsy at death. Specific exclusion criteria—including Parkinsonism, active substance use disorder, and severe neuropsychiatric disorder (e.g., bipolar disorder or schizophrenia)—were applied before recruitment, but participants who developed these conditions while in the study remained in the cohort. All UK-ADRC study activities are approved by the University of Kentucky Institutional Review Board, and participants provide written informed consent.

The UK-ADRC cohort includes 1033 participants who passed away from September 1st, 1990, to January 1st, 2024 and underwent autopsy. Because participation in ADRD clinical trials within this cohort began in 2005, we standardized our dataset by including only individuals (both trial participants and trial-naive participants) who died from 2005, to January 1st, 2024. Participants were initially categorized into two groups: those who engaged in ADRD clinical trials (n = 67) and those who were trial-naive (n = 546). We assessed whether the clinical trial cohort was representative of the total autopsied sample by comparing the demographics, genetic, and clinical measures, including age at death, sex, years of education, participants from underrepresented racial and ethnic groups, and the last study visit prior to death scores of MMSE CDR global (CDR-G) and CDR sum of boxes (CDR-SB) in addition to *APOE* genotype (any ε4 vs. no ε4).

We subdivided the 67 trial-engaged participants into three groups based on the types of clinical trials they participated in. The AD prevention trial (P) category included interventions for the majority of the study subjects were cognitively intact at the time of enrollment and participated in interventions aimed at preventing the future development of dementia (n = 21). The vascular trial (V) category included participants with vascular risk factors (e.g., hypertension, hyperlipidemia, DM, CVA, etc.), either subjective memory complaints, mild cognitive impairment (MCI), or mild dementia consistent with a diagnosis of VCID (n = 20). Though non-pharmacological trials, these V trials were included because the aims were focused on characterizing biomarkers of CVD. Lastly, the MCI/Dementia trial with presumed etiology of AD (MCI/D) category included participants diagnosed with MCI or Dementia at the time of enrollment in the clinical trials listed in Table 1 (n = 26).

2.2. Neuropathologic classification

Autopsies included the neuropathological assessments specified in the National Alzheimer's Coordinating Center (NACC) Neuropathology Data Set protocol [20]. The present analysis focused on amyloid-β, tau, α-synuclein, TDP-43 status, and CVD using consensus-based semi-quantitative rating scales [21]. For analysis, we dichotomized the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuritic amyloid plaques scores as 0 (CERAD None and CERAD Possible AD) and 1 (CERAD Probable AD and CERAD Definite AD). Similarly, Braak neurofibrillary tangle (NFT) stages were dichotomized as 0 (Braak NFT stages 0 - II) and 1 (Braak NFT stages III-VI). TDP-43 pathology for limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC) [22] was classified as present (LATE score 1 and above) or absent (yes = 1/no = 0). Alpha-synuclein staining for Lewy body pathologies [23] was also scored as present and absent (yes = 1/no = 0). CERAD neuritic plaque scores were used as the primary measure of amyloid-β pathology, as neurotic plaques reflect active amyloid-associated neuronal injury most directly relevant to the disease mechanisms targeted by the interventions studied. CVD pathologic scores included multiple vascular pathologies, namely: 1. Any infarcts scored as 0 for no infarcts, 1 for a single infarct and 2 for ≥ 2 infarcts, to account for the greater contribution of multiple infarcts to overall VCID pathology burden. 2. Atherosclerosis scored as 0 (none or mild), and 1 (moderate or severe); 3. Global cerebral amyloid angiopathy (CAA) scored as 0 (none or mild CAA), and 1 (moderate or severe); and 4. Global brain arteriolosclerosis scored as 0 (none or mild), and 1 (moderate or severe). CAA has known links to Aβ biology but was classified as a vascular small-vessel pathology per NIA–AA/NACC conventions [7,20,24,25] The classification of significant CVD was applied to any cases that have two or more out of 4 CVD pathologic features described above (maximum possible score = 5).

Table 1
Clinical trials represented in the autopsy cohort, including trial acronym, proposed mechanism, target population, and number of participants contributed by each trial.

Acronym	Brief Description	N	Proposed Mechanism	Population Targeted	NCT[#]
AWARE	ABBV-8E12 in Early AD	1	Tau-Targeting	MCI/Dementia	NCT02880956
CONNECT	Azeliragon (TTP488) therapy in Mild AD	6	RAGE inhibitor	MCI/Dementia	NCT02167256
IGIV	IGIV 10 % Treatment for Mild-to-Moderate AD	5	Immunomodulation	MCI/Dementia	NCT00818662
SNIFF	Nasal Insulin in the Dementia	3	Insulin signaling	MCI/Dementia	NCT01767909
ELAN	AN1792 active immunotherapy for AD	1	Immunomodulation	MCI/Dementia	NCT00088673
SUVEN-502	SUVN-502 +Donepezil + Memantine in Moderate AD	2	5-HT6 antagonist	MCI/Dementia	NCT02580305
TCAD (US202)	T-817MA in Mild to Moderate AD	5	Neuroprotection	MCI/Dementia	NCT02079909
STEADFAST	Azeliragon (TTP488) in Patients with Mild AD	1	RAGE inhibitor	MCI/Dementia	NCT02080364
DHA	DHA supplementation in Mild AD	1	Neuroprotection	MCI/Dementia	NCT00440050
T2 Protect AD	BHV-4157 in mild to moderate AD	1	Glutamate modulation	MCI/Dementia	NCT03605667
A4[#]	Solanezumab for Older Individuals Who May be at Risk for Memory Loss	2	Amyloid clearing	Prevention (2°)	NCT02008357
Alpha Phase-1*	The Antioxidant AT-001 for Reducing Brain Oxidative Stress	3	Antioxidant	Prevention (1°)	NCT01731093
Alpha Phase-2*	AT-001 for Long-term Preservation of Brain Health in Alzheimer's disease	1	Antioxidant	Prevention (1°)	NCT03062384
Gemfib*	Gemfibrozil in Predementia AD	8	MiRNA modulation	Prevention (1°–2°)	NCT02045056
INCREASE	Cognitive Reserve Enhancement in Delaying AD onset	4	Cognitive reserve	Prevention (1°)	NCT02849639
VPA	Valproic Acid intervention for Intact Cognition	3	Neuroprotection	Prevention (1°)	NCT01729598
MCI-CVD	Early Detection and Prevention of Mild Cognitive Impairment Due to Cerebrovascular Disease	18	CVD biomarker characterization	VCID	NCT01924312
MARK-VCID	Biomarkers for Vascular Contributions to Cognitive Impairment and Dementia Consortium	2	CVD biomarker characterization	VCID	NCT06284213

*Indicates the trials targeted by the majority of the normal population and few MCIs. [#]indicates A4 trial is the only trial requiring AD biomarkers (Amyloid positivity) for inclusion/exclusion. 1°, Primary, 2° Secondary prevention. Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-beta; MCI, mild cognitive impairment; VCID, vascular cognitive impairment and dementia; RAGE, receptor for advanced glycation end products; DHA, docosahexaenoic acid; miRNA, microRNA; CVD, cerebrovascular disease; APOE, apolipoprotein E; IGIV, intravenous immunoglobulin; VPA, valproic acid, NCT Clinicaltrials.gov identifier.

The on-target/off-target classification used in this study was based on autopsy confirmed neuropathology relative to the intended biological target of each clinical trial intervention rather than biomarker eligibility criteria or whether the participant met the original preventive objective of the trial. On-target pathology was defined as the presence of only those pathologic features inherent in the disease state targeted by the intervention in each clinical trial. This included amyloid-β, with or without tau for MCI/D trials; either no pathology or amyloid-β, with or without tau pathology for P trials, and only CVD pathology meeting significant criteria for the V trials. Alpha-synuclein and TDP-43 pathology were not considered part of on-target pathology, as no interventions in the included trials were designed to target these proteinopathies. Off-target pathologies were defined as neuropathologic features outside the intended biological target of the intervention.

2.3. Statistical analysis

Standard descriptive statistics, including means, standard deviations, and frequencies, are presented. Comparative statistics included the Welch *t*-test significant for between group analyses. All trial-engaged participants were further compared on demographic variables and ApoE4 status between cases that had only on-target vs. those with comorbid or exclusive off-target pathologies. The effects of time lag between clinical trial engagement and eventual death were tested with a Spearman correlating study. The p-value for statistical significance was set at <0.05 for this exploratory study. Few data points were missing on demographics. Those data points were not included in the analysis.

3. Results

Demographic, genetic, and clinical characteristics are presented in Table 2. Trial-engaged participants did not differ significantly from the trial-naïve group for demographic, genetic, and clinical characteristics with the exception of education which was marginally higher in the trial-engaged group ($p < 0.05$).

Given the variable time lag between clinical trial engagement and eventual death, we examined whether the duration of time from clinical trial participation to eventual death and autopsy influenced the number of pathologic features seen. Spearman correlation analysis revealed no significant association ($\rho = 0.172$, $p = 0.16$), suggesting that the duration between trial engagement and death does not significantly impact the extent of pathology accumulation. As none of the interventions used in this cohort are known to modify AD pathology, treatment exposure was not considered to influence these autopsy findings.

Table 2
Demographic, clinical, and genetic (APOE ε4 carrier status) characteristics of trial and non-trial participants in the UK autopsy cohort.

	Trial Participants (N)	Non-trial Participants (N)
Age at death years	81.9 ± 9.7 (67)	84.2 ± 9.8 (546)
Education years*	16.3 ± 3.2 (67)	15.4 ± 3 (505)
Male Sex (%)	52 % (67)	52 % (546)
Participants from underrepresented racial/ethnic groups, n (%)	4.5 % (67)	3.1 % (509)
MMSE total score (mean ± SD)	20.4 ± 8.8 (63)	20.0 ± 9.5 (489)
CDR global score (mean ±SD)	1.1 ± 1.1 (67)	1.3 ± 1.2 (488)
CDR sum of boxes (mean ± SD)	6.3 ± 6.4 (67)	7.30 ± 7.012 (488)
Apo ε4 carrier, n (%)	46.8 % (62)	39.6 % (497)

Data are presented as mean ± standard deviation (SD) or percentage, as appropriate. Continuous variables were compared using Welch's two-sample *t*-test. Only available data were included in each analysis; therefore, sample sizes vary across variables and are indicated in parentheses. Abbreviations: MMSE, Folstein Mini-Mental State Examination; CDR, Clinical Dementia Rating Scale; ApoE4, apolipoprotein E4 allele.
* $p < 0.05$.

The proportion of cases exhibiting only on-target pathology was 10 % in V, and 23.1 % in MCI/D groups (Fig. 1). Cases that exhibited only on-target pathology for the P group were seen at a much higher frequency of 28.6 %. Although on-target pathology was defined for P group as the presence of either no pathology or amyloid-β, with or without tau pathology, all these 28.6 % of participants had no detectable pathology at autopsy. Notably, there were no participants in the P group who exhibited only amyloid-β, with or without tau pathology (Fig. 1). This finding can be interpreted in two ways: either the intervention agents

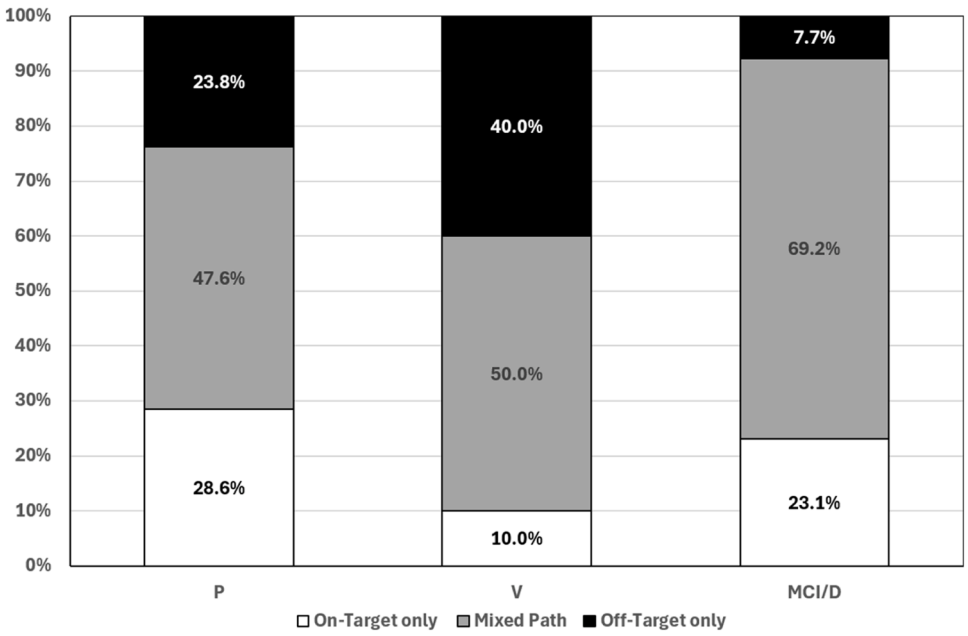

Fig. 1. Frequency of On-target/mixed/off-target pathologic features in each trial type. Trial type classification of participants based on having only the trial-targeted pathology vs. additional co-morbid off-target pathologic features (mixed) vs. those with only off-target pathologic features demonstrates a high prevalence of off-target comorbid pathologic features that may confound targeted disease modifying therapy trials. Abbreviations: MCI, mild cognitive impairment; D, dementia of the Alzheimer-type; path, pathologic feature(s).

were effective in preventing the development of AD pathology in this group, or these individuals may never have been predisposed to develop AD pathology, regardless of the intervention. Because none of the prevention-trial interventions have been shown to clear amyloid or prevent AD pathology, the absence of pathology at autopsy cannot be interpreted as a treatment effect and may simply reflect underlying biological predisposition.

The frequency of cases that did not exhibit any on-target pathology, but only off-target pathologic features, was 23.8 % for P, 40 % for V, and 7.7 % for MCI/D trial participants (Fig. 1). These cases only included off-target pathological features such as α- Synuclein, TDP-43/LATE-NC, and CVD pathologies where applicable.

The frequency of off-target pathologic features for participants without on-target pathology was 71.4 % for P, 90 % for V, and 76.9 % for MCI/D trials, respectively, which included cases irrespective of presence or absence of on-target pathology (Gray & Black bars in Fig. 1).

The frequency of comorbid pathologic features by trial type is summarized in Fig. 2A, which displays the percentages of participants with 0, 1, 2, 3, 4, or 5 pathologic features in each trial type. In the P and V trials, 28.6 % and 15.0 % of participants, respectively, had 0 features that met our operationalization threshold. In contrast, there were no participants in the MCI/D trial group without at least one of the studied pathologic features. Comorbid pathologic features were common, with 3 or more pathologic features seen in 52.3 % of P, 45 % of V, and 69.4 % of MCI/D trial participants. The average number of pathologic features per person across all trial participants was 2.54. When analyzed by group, the mean number of pathologic features per person was 2.1 in the P group, 2.54 in the V group, and 3.19 in the MCI/D trial-engaged group.

Fig. 2B highlights amyloid-β and tau pathologies in the MCI/D trial participants (92 % and 96 %, respectively) as expected but were also seen at high frequencies in the P (48 % and 52 %, respectively), and V trial participants (35 % and 65 %, respectively). Comorbid α-synuclein pathology was present in 14 %, 35 %, and 38 % of P, V, and MCI/D trial participants, respectively. Comorbid TDP-43 pathology was also common at 29 %, 25 %, and 46 % in P, V, and MCI/D trial participants, respectively. Vascular pathology was the most common comorbid pathology in the P as 62 %. Vascular and TDP were equivalently seen most common off-target pathologies (46 %) seen in MCI/D trial participants.

In an exploratory study (given the small sample sizes), we investigated whether demographic characteristics and *APOE* status could help refine inclusion/exclusion (I/E) criteria to minimize the confounding effects of comorbid or exclusively off-target pathologies (Table 3). Participants with on-target pathology alone were younger at death than those with mixed neuropathology (77.9 vs. 83.4 years, $p = 0.027$), whereas education, APOE genotype, and the remaining clinical characteristics did not differ significantly between groups.

4. Discussion

In this retrospective study, the frequencies of comorbid pathologies across clinical trial types demonstrate a high prevalence of comorbid pathologies. This data demonstrates that mixed pathology, representing multi-etiology dementia, is the norm rather than the exception in ADRD clinical trials. TDP-43, α-synuclein, and a moderate to severe degree of vascular pathology were frequently seen across clinical trial types. These findings demonstrate that clinical trial participants demonstrate similar profiles of mixed comorbid pathologic features compared to those seen in broader observational and cohort study populations investigating AD/ADRD [7,26,27], and further highlight the need to account for these complexities in therapeutic development and trial design.

While rigorous clinical trial I/E criteria are designed to enhance safety and efficacy, maximize interpretation of study results, and limit confounds that may interfere with safety and efficacy outcomes, the present study indicates that these measures are challenged by the frequency of comorbid pathologies in the community. Given the limited success with recent disease-modifying drug trials demonstrating only a 22–40 % slowing of clinical outcomes with complete or marked removal of amyloid pathology from the brain, the field is struggling to explain the disconnect between biological versus clinical efficacy [28]. Several explanations have been proposed, including the confound of mixed pathologic features, which the present manuscript highlights [11,29]. Many participants already have substantial tau pathology by the time symptomatic anti-amyloid trials begin, and tau burden is strongly linked to cognitive decline. Thus, even effective amyloid reduction may have

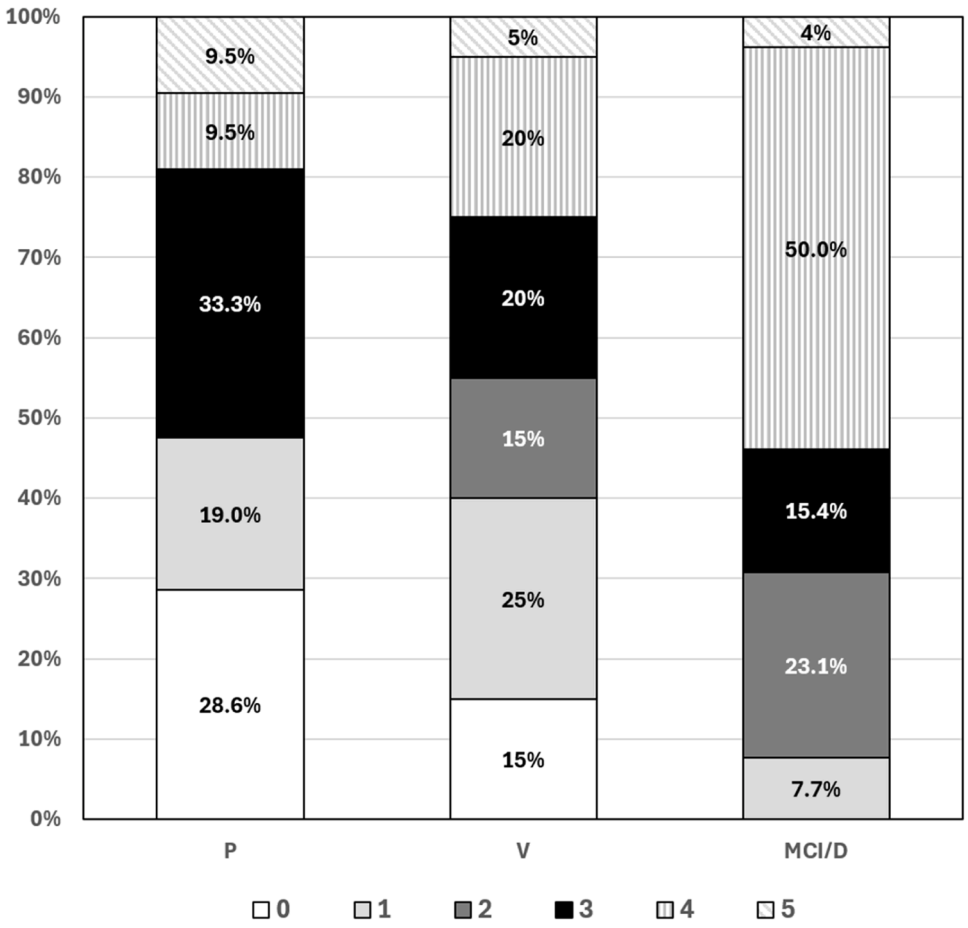

Fig. 2. Participant autopsy findings illustrate the number (Panel A) and complexity (Panel B) of comorbid pathologies seen in participants by discrete trial types. **Panel A** demonstrates clinical trial participants have more than one pathologic feature, highlighting the need for either improved screening measures or outcome measures and data analysis plans that take this complexity into account. The mean number of pathologic features per participant across study groups was calculated. The overall average pathology count was 2.54 features per person. Group-specific means were 2.05 in the P group, 2.54 in the V group, and 3.19 in the MCI/D trial-engaged group. **Panel B** demonstrates that comorbid Lewy Body disease, and TDP-43 proteinopathy confound both Alzheimer prevention and early-stage Alzheimer treatment trials. This data further demonstrates that Alzheimer pathology confounds clinical studies of cerebrovascular disease and vice-versa. Abbreviations: MCI, mild cognitive impairment; D, dementia of the Alzheimer-type; Aβ, β-amyloid plaques; Tau, Braak stage; α-Syn, Alpha-Synuclein for Lewy body pathology; TDP, Tar-DNA binding protein 43.

limited clinical impact once tau pathology is established, interacting with, rather than replacing, the effects of non-AD comorbidities.

Mixed pathologic features probably reflect distinct neurodegenerative or CVD processes, which may be unrelated to the various study interventions in targeting and abrogating any one distinct pathologic process. With the potential and in-progress development of new biomarkers for common comorbid pathological features, such as TDP-43, α-synuclein, and CVD [30–33], I/E criteria might focus on not just confirming a targeted biological process, but also on excluding comorbid neurodegenerative processes responsible for multi-etiology dementia. Simply excluding participants based on clinical labels like Parkinsonian syndromes or CVD has proven insufficient in eliminating underlying comorbid pathologies that may drive current or future cognitive decline, regardless of the primary clinical diagnosis or the on-target efficacy of the investigational drug.

In our exploratory analysis, participants with mixed neuropathology were older at death than those with on-target pathology alone, whereas education, APOE genotype, and other clinical characteristics did not differ significantly. One possible explanation is that individuals surviving to older ages have greater opportunity to accumulate additional age-related neuropathologies, including cerebrovascular disease, α-synuclein, and TDP-43 pathology, resulting in a mixed neuropathologic phenotype. Alternatively, individuals with relatively pure AD pathology may develop symptomatic disease earlier, leaving less time for the accumulation of additional age-related neuropathologies. Because of the limited sample size, these findings should be considered hypothesis-generating and require confirmation in larger autopsy cohorts.

The present data also highlights that parameters such as demographics and *APOE* genotype may not prove to be of much help in refining I/E criteria to eliminate the confounds inherent in common comorbid mixed pathologic features. Indeed, limiting I/E criteria for this purpose based on demographics and/or genetics may only serve to limit the generalizability of the data gathered in any particular trial. Refining I/E criteria must strike a balance between achieving a more homogeneous trial population and maintaining the generalizability of the trial results. Overly restrictive criteria could exclude many real-world patients who might otherwise benefit from such therapies, limiting the external validity of the findings. These data drive home the point that the confounding influence of mixed comorbid pathological features is an important, if not critical, concern, and further suggest that such study design challenges may be ameliorated through proactive clinical trial design improvements, such as refinement of I/E criteria. Although APOE did not aid in distinguishing mixed neuropathologies in this cohort, it remains important for risk stratification, trial safety considerations

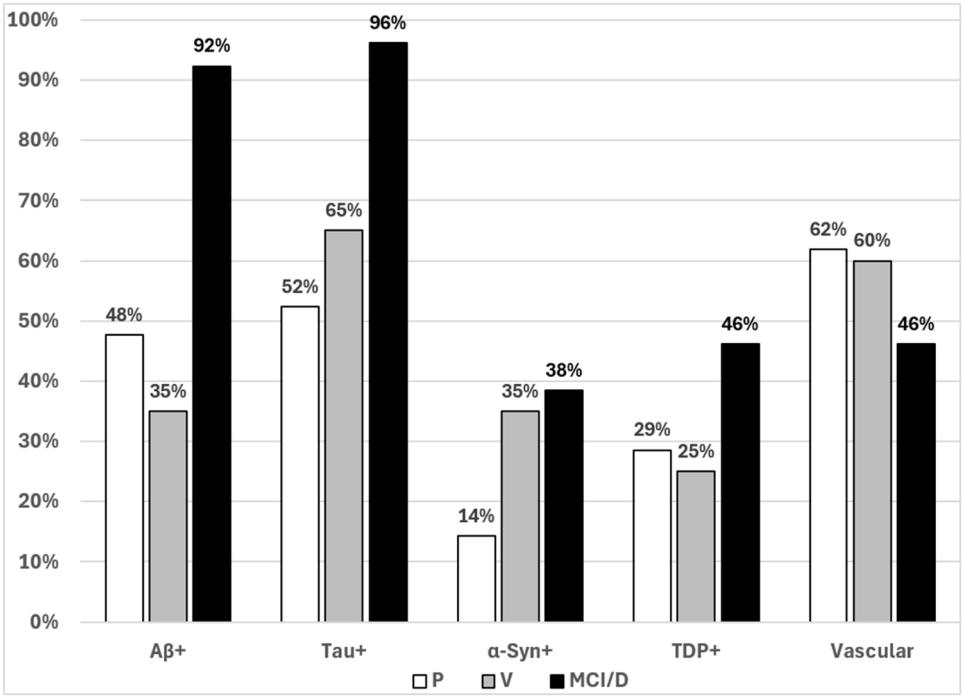

Fig. 2. (*continued*).

Table 3
Demographic, clinical, and genetic characteristics of participants with on-target versus mixed neuropathology.

Variable	On-Target Pathology (N)	Mixed Pathology (N)
Age at death, years *	77.7 ± 8.68(18)	83.4 ± 9.62 (49)
Education, years	15.9 ± 2. (618) (11)	16.4 ± 3.4 (49)
Male Sex (%)	61.1 % (18)	49.0 % (49)
Participants from underrepresented racial/ethnic groups, n (%)	1.7 % (18)	34.1 % (49)
Apo ε4 carrier, n (%)	52.9 % (17)	44.4 % (45)

On-target pathology included no pathology or amyloid-β, with or without tau pathology for prevention trials, amyloid-β with or without tau pathology for MCI/AD trials, and only vascular pathology for vascular trials. Mixed pathology includes on-target pathology together with one or more off-target neuropathologies. Data are presented as mean ± standard deviation (SD) or percentage, as appropriate. Only available data were included in each analysis; therefore, sample sizes vary by variable and are indicated in parentheses.

* $p < 0.05$.

(particularly ARIA risk in anti-amyloid trials), and interpretation of biomarker and disease-progression patterns.The present data also illustrates the importance of considering antemortem biomarker status in developing I/E criteria; only 7.7 % of participants in MCI/D trials lacked the targeted pathology. This frequency was larger prior to the development of accurate antemortem biomarkers for detecting disease pathology that is the target for trial intervention. Indeed, in comparison to most current amyloid-oriented clinical trials, the early solanezumab studies (that did not require amyloid-β positivity by PET for inclusion) had a much higher frequency of participants lacking the amyloid-β pathology [34]. Most, but not all the participants included in the present study were in studies that required antemortem biomarker positivity for inclusion. This likely contributed to the low frequency of participants for whom the intervention was unlikely to be effective based on their underlying pathology. Correspondingly, our data strongly argue for the inclusion of antemortem biomarkers to identify on-target pathology when available. Prevention trials were grouped together as compared to other trial groups because individual trials in each group lacked biomarker confirmation and had very small autopsy samples, making further subdivision underpowered.

The present data demonstrating the high prevalence of comorbid pathologies for participants in clinical trials reinforces the idea that neurodegenerative disease states are complex and heterogeneous [12,35]. While many AD/ADRD clinical trials incorporated antemortem biomarkers such as amyloid and tau PET imaging or CSF-based markers to identify on-target pathology, few included comprehensive screening for other common co-pathologies, such as VCID, LATE-NC, and α-synucleinopathies [36,37]. Of course, there are challenges for each of these: we do not yet have an antemortem biomarker for TDP-43 pathology [38], whereas α-synuclein biomarkers are in the earliest stages of clinical development and use [39], and antemortem biomarkers of CVD pathology while available, largely through MRI measures of white matter hyperintensities [40], have not yet been thoroughly vetted and established cut points have not been agreed upon. The absence of standardized biomarker-based inclusion criteria for such (often co-morbid) pathologic features has likely influenced the results of many past trials, leading to variable treatment responses and overall negative trial conclusions, even for some interventions that might have proven successful otherwise. Moving forward, a more comprehensive, practical biomarker framework is needed to improve participant selection and trial stratification.

Developing a full armament of antemortem biomarkers for the common pathologic comorbidities seen in ADRD trials will also enhance our ability to begin designing a precision-medicine approach to clinical trials in ADRD [41,42]. Such approaches may consider multi-targeted interventions and/or combination therapies to address common co-morbid pathologic disease states. The development and implementation of combination therapy approaches could offer a promising solution for tackling multiple disease mechanisms. Recent advancements [43] in fluid biomarkers and neuroimaging, and approved therapies that reduce amyloid pathology, offer promising approaches [4,6], which would facilitate the development of such precision-medicine approaches involving combination therapeutic approaches for improved ADRD trial success.

This study has limitations that should be considered when interpreting the results. Firstly, the participant cohort largely consisted of Caucasian individuals with higher educational levels that were willing to undergo autopsy, reflecting a selection bias that limits the generalizability of the present findings [44,45]. Conversely, such discovery could not be achieved if such a select group of individuals did not agree to engage in all aspects of research from observational studies to clinical trials, to eventual autopsy. The small sample size is also a weakness of the present study. Overcoming barriers to engagement through educational initiatives and reinforcing the need for eventual autopsy confirmation is in its infancy in the setting of clinical trial research [46–48]. The NIH-funded Alzheimer Clinical Trial Consortium (ACTC) has embraced this imperative with an active autopsy registry program specifically targeting clinical trial participants. The present data argue for more widespread engagement in such long-term programs across clinical trial sponsors including not just Federal, but also non-profit and Pharma sponsors [38,49,50]. Ultimately, further research and investment are needed to tackle the challenges inherent in ADRD clinical interventional research.

In conclusion, we found that multiple dementia-associated neuropathologies were the norm in participants who had been recruited for AD/ADRD clinical trials. Future work needs to focus not only on specificity for inclusion's sake, but also considerations should be made for stratifying based on risk for off-targeted pathologies. Furthermore, our study provides added evidence about the possible future utility of multiple therapeutic interventions in individual patients. The field needs to continue to focus on additional biomarker development and trial design innovations, and post-mortem tissue collection programs to further advance the present findings. Fortunately, such work is well underway across the globe as we move into a new era of precision medicine in ADRD clinical trial design, implementation, and therapeutic development.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used OpenAI's ChatGPT (GPT-5.5) for proofreading and language editing. The authors reviewed and edited the output as needed and take full responsibility for the content of the published article.

Funding

This study was also supported by NIH P30 AG072946 and NIH RO1 AG061111.

CRediT authorship contribution statement

E. Pinar Coskun: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Justin Barber:** Writing – review & editing, Methodology, Data curation. **Lauren Bojarski:** Writing – review & editing. **Christopher J. McLouth:** Writing – review & editing, Methodology, Data curation. **Erin L. Abner:** Writing – review & editing, Validation, Formal analysis, Data curation. **Linda J. Van Eldik:** Writing – review & editing, Resources. **Peter T. Nelson:** Writing – review & editing, Conceptualization. **Gregory A. Jicha:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would also like to acknowledge the participants and their family members whose contributions were invaluable to the data collected for this study. Without their dedication and support, this research would not have been possible. We would also like to acknowledge the countless hours, years, and decades of hard work invested by researchers in the development of therapeutic interventions for the disease that has paved the way for advancements in the field.

Data availability

Anonymized UK-ADRC data not published within this article, and not including proprietary clinical trial data, will be made available by request from any qualified investigator.

References

[1] (FDA), U.S.F.a.D.A. FDA Converts Novel Alzheimer's Disease Treatment to Traditional Application. FDA; 2023.
[2] (FDA), U.S.F.a.D.A., FDA approves treatment for adults with Alzheimer's disease. 2024.
[3] Cummings J, et al. Alzheimer's disease drug development pipeline: 2024. Alzheimer's Dement: Transl Res Clin Interv 2024;10(2).
[4] van Dyck CH, et al. Lecanemab in early Alzheimer's Disease. N Engl J Med 2023;388(1):9–21.
[5] Knopman DS, Hershey L. Implications of the approval of Lecanemab for Alzheimer Disease patient care: incremental step or paradigm shift? Neurology 2023;101(14):610–20.
[6] Sims JR, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. JAMA 2023;330(6):512.
[7] Schneider JA, et al. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. Neurology 2007;69(24):2197–204.
[8] Robinson JL, et al. Pathological combinations in neurodegenerative disease are heterogeneous and disease-associated. Brain 2023;146(6):2557–69.
[9] Karanth S, et al. Prevalence and clinical phenotype of quadruple misfolded proteins in older adults. JAMA Neurol 2020;77(10):1299.
[10] Mehta RI, Schneider JA. Neuropathology of the common forms of dementia. Clin Geriatr Med 2023;39(1):91–107.
[11] Tosun D, et al. Identifying individuals with non-Alzheimer's disease co-pathologies: a precision medicine approach to clinical trials in sporadic Alzheimer's disease. Alzheimers Dement 2024;20(1):421–36.
[12] Rabinovici GD, et al. Multiple comorbid neuropathologies in the setting of Alzheimer's disease neuropathology and implications for drug development. Alzheimers Dement (N Y) 2017;3(1):83–91.
[13] Gauthreaux K, et al. Concordance of clinical Alzheimer diagnosis and neuropathological features at autopsy. J Neuropathol Exp Neurol 2020;79(5):465–73.
[14] Nicoll JAR, et al. Persistent neuropathological effects 14 years following amyloid-beta immunization in Alzheimer's disease. Brain 2019;142(7):2113–26.
[15] VandeVrede L, et al. Co-pathology may impact outcomes of amyloid-targeting treatments: clinicopathological results from two patients treated with aducanumab. Acta Neuropathol 2023;146(5):777–81.
[16] Gilman S, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 2005;64(9):1553–62.
[17] Beach TG, Malek-Ahmadi M. Alzheimer's Disease neuropathological comorbidities are common in the younger-old. J Alzheimers Dis 2021;79(1):389–400.
[18] Schmitt FA, et al. University of Kentucky Sanders-Brown healthy brain aging volunteers: donor characteristics, procedures and neuropathology. Curr Alzheimer Res 2012;9(6):724–33.
[19] Smith VD, et al. Overlapping but distinct TDP-43 and tau pathologic patterns in aged hippocampi. Brain Pathol 2018;28(2):264–73.
[20] Besser LM, et al. The Revised National Alzheimer's Coordinating Center's neuropathology form-available data and new analyses. J Neuropathol Exp Neurol 2018;77(8):717–26.
[21] Montine TJ, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 2012;123(1):1–11.
[22] Nelson PT, et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. Brain 2019;142(6):1503–27.
[23] Attems J, et al. Neuropathological consensus criteria for the evaluation of lewy pathology in post-mortem brains: a multi-centre study. Acta Neuropathol 2021;141(2):159–72.
[24] Hyman BT, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 2012;8(1):1–13.
[25] Nelson PT, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol 2012;71(5):362–81.
[26] Rahimi J, Kovacs GG. Prevalence of mixed pathologies in the aging brain. Alzheimers Res Ther 2014;6(9):82.

[27] James BD, et al. TDP-43 stage, mixed pathologies, and clinical Alzheimer's-type dementia. Brain 2016;139(11):2983–93.
[28] Aljuhani M, Ashraf A, Edison P. Evaluating clinical meaningfulness of anti-beta-amyloid therapies amidst amyloid-related imaging abnormalities concern in Alzheimer's disease. Brain Commun 2024;6(6):fcae435.
[29] Banks SJ, et al. Enriching the design of Alzheimer's disease clinical trials: application of the polygenic hazard score and composite outcome measures. Alzheimers Dement (N Y) 2020;6(1):e12071.
[30] Shaw LM, et al. ADNI Biomarker Core: a review of progress since 2004 and future challenges. Alzheimers Dement 2025;21(1):e14264.
[31] Cao Y, et al. Fluid-based biomarkers for neurodegenerative diseases. Ageing Res Rev 2025;108:102739.
[32] Cordts I, et al. TDP-43 proteinopathy specific biomarker development. Cells 2023;12(4):597.
[33] Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. Mol Psychiatry 2021;26(1):296–308.
[34] Honig LS, et al. Trial of Solanezumab for mild dementia due to Alzheimer's disease. N Engl J Med 2018;378(4):321–30.
[35] Toledo JB, et al. Dementia with lewy bodies: impact of co-pathologies and implications for clinical trial design. Alzheimers Dement 2023;19(1):318–32.
[36] Jack Jr CR, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14(4):535–62.
[37] Toledo JB, et al. CSF alpha-synuclein improves diagnostic and prognostic performance of CSF tau and Abeta in Alzheimer's disease. Acta Neuropathol 2013;126(5):683–97.
[38] Wolk DA, et al. Clinical criteria for limbic-predominant age-related TDP-43 encephalopathy. Alzheimers Dement 2025;21(1):e14202.
[39] Magalhaes P, Lashuel HA. Opportunities and challenges of alpha-synuclein as a potential biomarker for Parkinson's disease and other synucleinopathies. NPJ Parkinsons Dis 2022;8(1):93.
[40] Vemuri P, et al. White matter abnormalities are key components of cerebrovascular disease impacting cognitive decline. Brain Commun 2021;3(2):fcab076.
[41] Arafah A, et al. The future of precision medicine in the cure of Alzheimer's disease. Biomedicines 2023;11(2):335.
[42] Behl T, et al. The road to precision medicine: eliminating the "one size fits all" approach in Alzheimer's disease. Biomed Pharmacother 2022;153:113337.
[43] Therriault J, et al. Diagnosis of Alzheimer's disease using plasma biomarkers adjusted to clinical probability. Nat Aging 2024;4(11):1529–37.
[44] Franzen S, et al. Diversity in Alzheimer's disease drug trials: the importance of eligibility criteria. Alzheimers Dement 2022;18(4):810–23.
[45] Raman R, et al. Tackling a major deficiency of diversity in Alzheimer's disease therapeutic trials: an CTAD Task Force report. J Prev Alzheimers Dis 2022;9(3):388–92.
[46] Glover CM, et al. Perceived impediments to completed brain autopsies among diverse older adults who have signed a uniform anatomical gift act for brain donation for clinical research. Ethn Dis 2020;30(Suppl 2):709–18.
[47] Langbaum JB, et al. Recommendations to address key recruitment challenges of Alzheimer's disease clinical trials. Alzheimers Dement 2023;19(2):696–707.
[48] Castonguay G, et al. Overcoming barriers to implementation of patient engagement in clinical trials: feasibility testing of an embedded study. Res Involv Engag 2025;11(1):15.
[49] Sachdev P, et al. Diagnostic criteria for vascular cognitive disorders: a VASCOG statement. Alzheimer Dis Assoc Disord 2014;28(3):206–18.
[50] Blevins BL, et al. Brain arteriolosclerosis. Acta Neuropathol 2021;141(1):1–24.