Paquet, C., Nicoll, J. A., Love, S., Mouton-Liger, F., Holmes, C., Hugon, J., & Boche, D. (2017). DOWNREGULATED APOPTOSIS AND AUTOPHAGY AFTER ANTI-A IMMUNOTHERAPY IN ALZHEIMER'S DISEASE. *Brain Pathology*. https://doi.org/10.1111/bpa.12567

DOWNREGULATED APOPTOSIS AND AUTOPHAGY AFTER ANTI-Aβ IMMUNOTHERAPY IN ALZHEIMER'S DISEASE

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/bpa.12567

Abstract

Aβ immunisation of Alzheimer's disease (AD) patients in the AN1792 (Elan Pharmaceuticals) trial caused Aβ removal and a decreased density of neurons in the cerebral cortex. As preservation of neurons may be a critical determinant of outcome after Aβ immunisation, we have assessed the impact of previous Aβ immunisation on the expression of a range of apoptotic proteins in post-mortem human brain tissue. Cortex from 13 AD patients immunised with AN1792 (iAD) and from 27 non-immunised AD (cAD) cases was immunolabelled for pro-apoptotic proteins implicated in AD pathophysiology: phosphorylated c-Jun N-terminal kinase (pJNK), activated caspase3 (a-casp3), phosphorylated GSK3β on tyrosine 216 ($GSK3\beta_{tyr216}$), p53 and Cdk5/p35. Expression of these proteins was analysed in relation to immunisation status and other clinical data. The antigen load of all of these pro-apoptotic proteins was significantly lower in iAD than cAD ($p < 0.0001$). In cAD, significant correlations ($p < 0.001$) were observed between: Cdk5/p35 and $GSK3\beta_{tyr216}$; a-casp3 and $A\beta_{42}$; p53 and age at death. In iAD, significant correlations were found between $GSK3\beta_{tyr216}$ and a-casp3; both spongiosis and neuritic curvature ratio and $A\beta_{42}$; and Cdk5/p35 and Aβ-antibody level. Although neuronal loss was increased by immunisation with AN1792, our present findings suggest downregulation of apoptosis in residual neurons and other cells.

Keywords: Alzheimer, treatment, anti-amyloid immunotherapy, brain, neurons, impact.

INTRODUCTION

Alzheimer's disease (AD) is characterized by the accumulation of β-amyloid (Aβ) peptide and hyperphosphorylated tau protein, and eventually synaptic and neuronal loss. The pathophysiology of the neuronal death remains unclear and controversial. Neuropathological studies have provided evidence of apoptotic neuronal death compatible with the slow progression of neuronal degeneration (15, 27, 32), in addition to possible deregulated autophagic activity (3, 14, 16, 24, 44). Apoptosis is a sequence of programmed events leading to the activation of caspases and cell disintegration (15, 27, 32), whereas autophagy is an intracellular catabolic process leading to the removal of aggregated proteins within cells (22, 28, 38). Both autophagy and apoptosis are highly regulated, play critical roles in tissue homeostasis, and tend to be upregulated in response to extracellular or intracellular stress and in neurodegenerative diseases (26). In AD, both processes have been extensively studied but their contribution to neuronal death remains unclear. Apoptotic cell death in AD may result from an imbalance between pro- and anti-apoptotic proteins (15). The expression of several pro-apoptotic kinases such as activated GSK3β phosphorylated at tyrosine 216 ($GSK3\beta_{tyr216}$) (1, 6, 37), pPKR (6, 7, 10, 29, 33, 34, 36), pJNK (9, 18, 42, 43), p53 (8) and activated caspase-3 (a-casp3) (2, 15, 17, 41) is increased in AD brains. In AD, autophagic activity is increased but may be dysfunctional, with failure of substrate clearance reflected by the presence of vacuoles (3, 14, 16, 24, 44).

Active $A\beta_{42}$ immunisation (AN1792, Elan Pharmaceuticals) in AD patients led to Aβ removal (19, 30, 31) associated with a decrease in phosphorylated tau (pTau) (4), long-term down-regulation of inflammation (46), reduction in the number of neurons and reduced neuritic abnormalities (34, 39). To investigate possible mechanisms underlying the observed neuronal loss after immunotherapy, we have explored the expression of apoptotic and autophagic proteins in the unique cohort of immunised AD patients from the AN1792 trial.

MATERIALS AND METHODS

Case selection

Immunised AD cases (iAD)

The brains of clinical AD patients enrolled in the initial Elan Pharmaceuticals Aβ immunisation trial AN1792 (19) were obtained following consent to *post-mortem* neuropathology. The study received ethical approval from Southampton and South West Hampshire Local Research Ethics Committees (Reference No: LRC 075/03/w). Thirteen *post-mortem* brains in which the cause of the dementia was confirmed as AD neuropathologically were included in this study. All patients had received $A\beta_{42}$ plus adjuvant and had died between 4 and 162 months after the first immunisation (mean 72.8 months, median 63 months), with Braak tangle stage V/VI disease, as previously described (34) (Table 1). The *post-mortem* delay was between 6 and 48 hours (mean 18.5 hours; median 6 hours). In addition to dementia, the most common clinical diagnoses recorded in the death certificate were bronchopneumonia, cerebrovascular accident and myocardial infarction. Other diagnoses included ruptured aortic aneurysm, pulmonary embolism, carcinoma of the breast, carcinoma of the bronchus, and carcinoma of the pancreas. Neurodegenerative pathology was assessed by standard histological methods including haematoxylin and eosin (H&E), Luxol fast blue/cresyl violet and modified Bielschowsky silver impregnation. Selected sections were immunolabelled for Aβ, tau, α-synuclein and TDP43 to confirm AD.

Non-Immunised AD cases (cAD)

Twenty-seven AD cases provided by the South West Dementia Brain Bank (SWDBB, Bristol, UK) were identified and used as a control unimmunised AD cohort (supplementary Table 1). All cAD cases had a clinical diagnosis of AD made during life by an experienced clinician, a Mini-Mental State Examination score of <17 prior to death and satisfied *post-mortem* neuropathological Consensus Criteria for Alzheimer's disease (20). The *post-mortem* delay was between 9 and 110 hours (mean 39 hours, median 26 hours). The immunised and control AD cases were matched as closely as possible for age, gender, duration of dementia and *APOE* genotype (Table 1). The SWDBB tissue was used

under the ethical approval from North Somerset and South Bristol Hampshire Local Research Ethics Committees (Reference No: REC 08/H0106/28+5).

Immunohistochemistry

Middle temporal gyrus, usually markedly affected by AD pathology, was investigated in this study. Four-µm sections of formalin-fixed paraffin-embedded tissue from iAD and cAD cases were immunolabelled together in batches to ensure comparability of staining.

Primary antibodies and immunohistochemistry

To evaluate the impact of active AN1792 immunisation on apoptotic and autophagic pathways, we explored by immunohistochemistry the expression of the following pro-apoptotic proteins: $GSK3\beta_{tyr216}$ (polyclonal rabbit anti-phosphorylated $GSK3\beta_{tyr216}$, #ab75745, Abcam) (6, 37), neuron-specific activator of cyclin-dependent kinase 5 with its activator p35 (C-19 polyclonal rabbit anti-Cdk5/p35, #sc-820, Santa Cruz) (12, 42), phosphorylated c-Jun N-terminal kinase (monoclonal rabbit anti-pJNK Thr183/Tyr185, clone 81E11, #4668, Cell Signaling) (18, 45), p53 (monoclonal mouse anti-p53, clone DO-1, #sc-126, Santa Cruz) (8), and a-casp3 (polyclonal rabbit anti-activated caspase 3 (Asp175), # 9661, Cell Signaling) (15, 40, 41); and of the autophagic proteins ATG5 (initial step) (polyclonal rabbit anti-ATG5, #AP1812b, Abgent) and microtubule-associated protein light chain LC3-II (a marker of the final stage reflecting efficient autophagic activity) (polyclonal rabbit anti- LC3-II, #AP1801a, Abgent) (21, 22, 28). The specificity of the antibodies pJNK (18), $GSK3\beta_{tyr216}$ (1), and CDK5/p35 (21) was previously demonstrated. In order to demonstrate the specificity of the antibodies p53, ATG5 and LC3II, we performed western blot on human brain tissue homogenates.

Immunohistochemistry was carried out by a standard method as previously described (1, 4, 5, 19, 30, 34, 46). Biotinylated secondary antibodies, normal serum and avidin-biotin complex were from Vector Laboratories (Peterborough, UK). Immunodetection was performed using the avidin-biotin-peroxidase complex method (Vectastain Elite ABC, UK) with 3,3'-diaminobenzidine (DAB) as chromogen and 0.05% hydrogen peroxide as substrate. All the sections were dehydrated before mounting in DePeX (BDH Laboratory Supplies, UK). Sections from which the primary antibody was omitted were included in each immunohistochemistry run.

Quantification of immunolabelling

Quantification was performed blind to the identity of the cases. Thirty fields of cortical grey matter at objective magnification x20 were acquired for each case from the same anatomical regions in a zigzag sequence along the cortical ribbon to ensure that all cortical layers were represented. Slides were marked by the same neuropathologist to ensure consistency in the location of acquisition of the images. Protein 'load' defined as the percentage of the field immunopositive for the marker of interest was determined using ImageJ (developed by W.S. Rasband National Institutes of Health, Bethesda MD, USA, version 1.47g), as in our previous studies (1, 4, 5, 19, 34, 46).

Statistical analysis

The normality of distribution of each marker across the cohort was assessed by examination of quantile-quantile plots (not shown). Levels of each marker were compared between cAD and iAD cases in two-sample two-sided t-tests or non-parametric Mann-Whitney U-tests (depending on the normality of the data). In both groups, correlations were analysed by Pearson's or Spearman's test, depending on the normality of distribution of the markers. We analysed the correlation between the apoptosis and autophagy-associated markers and (i) indicators of disease severity and neuronal integrity as reported in our previous published studies as follows: $A\beta_{42}$ load, pTau load, tangles density by image, dystrophic neurites, spongiosis, number neuronal NeuN+ density by image, neuritic curvature ratio assessed by neurofilament immunohistochemistry, phosphorylated (p)PKR (a marker of early neurodegeneration) (4, 19, 34, 46); and (ii) available clinical indicators of disease course and antibody response – duration of dementia, survival time after immunisation, age at death, mean and peak antibody level. The threshold for statistical significance was set at 5% for intergroup comparisons and 1% for correlations, as determined by use of SPSS 21.0.

RESULTS

The immunolabelling of all of the antigens was neuronal, with additional labelling of glial cells for some proteins as described in Table 2. Of note, the immunolabelling of activated-caspase 3 was cytoplasmic with the nuclei of the stained neurons morphologically normal, without the karyorrhexis classically associated with apoptosis.

The expression of all apoptotic kinases was significantly lower in iAD than cAD cases: a-casp3 load, $P<0.001$; Cdk5/p35 load, $P=0.013$; p53 load, $P<0.001$; GSK3β_{tyr216} load, $P<0.01$; and pJNK, $P<0.001$ (Figure 1). Of the two autophagic markers examined, LC3-II load was significantly lower in iAD than cAD ($P<0.001$) while ATG5 load did not differ between the cohorts ($P=0.130$, Figure 1).

The expression of apoptotic and autophagic markers was analysed for correlation with other aspects of AD pathology (Aβ42 load, pTau load, dystrophic neurite counts, spongiosis, NeuN+ neurons and curvature ratio) in the same anatomical region, and also with a range of clinical parameters (age, gender, age at death, dementia duration, peak antibody, survival time). We did not observe any modification in the distribution of the proteins between both cohorts except for the GSK3β_{tyr216}, which was detected mainly in granulo-vacular degeneration (GVD) in the iAD group but not in the cAD group. To take account of possible variations in neuronal density, we also assessed the percentage of all neurons that was immunopositive for a-casp3. This confirmed the striking decrease in neuronal expression of a-casp3 in iAD compared with cAD ($p<0.0001$, data not shown).

In the cAD group, a-casp3 load correlated positively with Aβ42 ($r=0.561$, $P=0.005$), and Cdk5/p35 correlated positively with pGSK3β_{tyr216} ($r=0.642$, $P<0.001$) (Table 3). Comparison of present findings with the clinical data revealed positive correlations between p53 and age at death ($r=0.564$, $P=0.003$), and between LC3-II and dementia duration ($r=0.691$, $P=0.001$) (Table 3).

Within the iAD cohort, a-casp3 and GSK3β_{tyr216} correlated positively with severity of spongiosis, a marker of neuropil degeneration ($r=0.789$, $P=0.004$ and $r=0.761$, $P=0.007$ respectively) (Table 2). ATG5 correlated negatively with Aβ42 load ($r=-0.845$, $P=0.001$) and positively with the curvature ratio (abnormal tortuosity of neuritic processes) ($r=0.841$, $P=0.001$) (Table 4). Cdk5/p35 correlated

positively with peak antibody titre (r=0.840, P<0.001) as well as with mean antibody titre (data not shown) (Table 4).

No other correlation was observed in either group.

DISCUSSION

Our results suggest that active Aβ immunisation of AD patients modulates apoptosis and some autophagic cellular signals, causing downregulation of apoptotic proteins and reduction in the final stage of autophagy activity. The decrease of apoptotic protein expression after immunisation could have several explanations: 1) Downregulation of apoptosis was a consequence of removal of Aβ, consistent with several studies implicating Aβ-induced apoptosis in neuronal death in AD (6, 8). 2) The reduction in apoptotic proteins may simply reflect the accelerated loss of damaged neurons after immunotherapy, as previously reported by us (34), potentially leaving 'healthier' neurons less affected by AD pathophysiology. However, the small magnitude of neuronal loss after immunotherapy (about 10%) could not be the sole explanation for the substantial decrease in apoptotic protein load (between 65% and 85%), and analysis of the percentage of all neurons that was immunopositive for a-casp3 confirmed the marked reduction in neuronal expression of this antigen in iAD. 3) Immunotherapy may itself down-regulate apoptotic proteins. Further studies are needed to clarify the cellular and molecular processes that underlie these findings.

The effects of autophagic proteins are less clear-cut. The reduction in LC3II suggests downregulation of the later steps of autophagy, potentially explained by reduced metabolic requirement for autophagy or perhaps an aborted or dysfunctional autophagic process. Restrictions on tissue availability did not allow us to explore this mechanistically. Analysis in animal models may help to clarify the influence of immunotherapy on autophagy.

The correlation between a-casp3 and $A\beta_{42}$ in the cAD group, is in accordance with previous reports implicating $A\beta_{42}$ in neuronal apoptosis (6, 15). The link between Cdk5/p35 with $GSK3\beta_{tyr216}$ is also

consistent with previous studies implicating these proteins in the pathophysiology of AD, particularly in the phosphorylation of Tau protein (13, 23).

Strikingly different associations were observed in the immunised cohort. The relationship between a-casp3, GSK3β_{tyr216} loads and the severity of spongiosis, a marker of neuropil degeneration, strengthen the association between these pro-apoptotic proteins and the neuronal loss detected after immunisation (34). This may explain the absence of clinical amelioration in these patients (19). Due to the nature of the post-mortem study, investigating late-stage of the disease and treatment, we cannot exclude the possibility that immunotherapy may have induced an early acute apoptotic phase followed by a more quiescent phase several years after the treatment.

The relationship between p53 expression and age at death in the control Alzheimer's cohort is consistent with the documented association between apoptosis and increasing age (11). The increase in LC3-II with dementia duration may be part of a pro-survival adaptive response by neurons and glia to minimise neurodegeneration (14). After immunisation, the anti-Aβ immune response (mean and peak Aβ antibody titre) was strongly associated with Cdk5/p35 expression. Cdk5/p35 signalling is known to promote microglial phagocytosis of fibrillar Aβ (25), and the present data are in keeping with the enhanced Aβ clearance by phagocytic microglia in the immunised patients who developed an immune response (19, 35, 46). However, it should be noted that the highest Cdk5/p35 level in the immunised cohort was much lower than that in the control group, consistent with the down-regulation of microglial activation that occurs when Aβ has been completely removed (46).

This study has some limitations, inherent in the use of post-mortem tissue. As previously reported (1, 4, 5, 19, 30, 34, 46), the number of placebo immunisation cases from which brains could be obtained (n=1) was far too low to provide useful data for statistical analysis and thus our study used AD brains from patients who were not included in a protocol of immunotherapy, although they were matched as closely as possible to the immunised cohort. Furthermore, this was a retrospective observational study rather than a prospective experimental study, which limited the range of methodological approaches and the comparability of clinical findings. Because this was an end-stage study, it was not possible to

explore the temporal relationship between markers of apoptosis or autophagy and neuronal loss, and analysis was limited to assessment of the late-stage consequences of immunisation.

In summary, in this unique human brain series from the first anti-$A\beta_{42}$ trial, our results suggest that anti-$A\beta_{42}$ immunisation downregulates the expression of several pro-apoptotic proteins in the brain. Whilst these changes might be expected to be beneficial, the absence of cognitive benefit suggests that they occur too late in the disease process or that other mechanisms are responsible for the neuronal death.

Abbreviations

a-casp3	activated caspase 3
AD	Alzheimer's disease
ATG5	autophagy-related gene 5
Aβ	β-amyloid
CDK5	cyclin dependent Kinase 5
$GSK3\beta_{tyr216}$	glycogen Synthetase Kinase 3 phosphorylated at tyrosine 216
iAD	immunised Alzheimer's Disease brains
JNK	c-Jun N Terminal Kinase
LC3	microtubule-associated protein light chain 3
p53	tumor protein 53
PKR	double-stranded RNA dependent protein kinase
iAD	immunised Alzheimer's disease brains
cAD	non-immunised Alzheimer's disease brains
pTau	phosphorylated tau

Ethical approval and consent to participate

The study received ethical approval from the Southampton and South West Hampshire Local Research Ethics Committees, Reference No. LRC 075/03/w for the use of the iAD cohort. The cAD cases were provided under the SWDBB Ethics (Research Ethics Committee Reference No. 08/H0106/28+5).

Competing interest

Prof. PAQUET is member of the International Advisory Boards of Lilly and is involved as investigator in several clinical trials for Roche, Esai, Lilly, Biogen, Astra-Zeneca, Lundbeck

Prof. NICOLL is or has been a consultant/advisor relating to Alzheimer immunisation programmes for Elan Pharmaceuticals, GlaxoSmithKline, Novartis, Roche, Janssen, Pfizer, Biogen.

Prof. HUGON is investigator in several passive anti-amyloid immunotherapies and other clinical trials for Roche, Eisai, Lilly, Biogen, Astra-Zeneca, Lundbeck.

Prof LOVE, Prof HOLMES, Dr BOCHE and Dr MOUTON-LIGER declare that they have no conflict of interest.

Funding

This study was supported jointly by the Fondation Philippe Chatrier (Paris, France), Alzheimer Research UK (ART/PG2006/4 and ART-EXT2010-1) and Medical Research Council UK (G0501033).

Author's contributions

Claire PAQUET designed the study, performed the immunohistochemistry experiments, collected and analysed the data and prepared the manuscript.

Delphine Boche analysed and interpreted the data and prepared the manuscript.

Seth Love provided the cAD cases from SWDBB and was involved in the preparation of the manuscript.

Clive Holmes provided the clinical data.

François Mouton-Liger performed Western blot to control for the specificity of the antibodies and prepared the manuscript.

Jacques Hugon advised on the relationship between different apoptotic kinases in Alzheimer's' disease.

James Nicoll provided immunised AD brains and was involved in the preparation of the manuscript.

All co-authors provided input and critically revised the paper.

"All authors read and approved the manuscript."

ACKNOWLEDGMENTS

All authors had full access to all data and CP and DB have final responsibility for the decision to submit the report for publication.

We thank the patients who were involved in this study and their careers. We thank all donors, the president and scientific committee of Fondation Philippe Chatrier. Vivienne Hopkins, David Wilkinson, Anthony Bayer, Roy Jones and Roger Bullock enrolled patients in the original trial. Jim Neal provided 2 immunised cases from Cardiff. We would like to thank the South West Brain Dementia Brain Bank (SWDBB) for providing tissue for this study. The SWDBB is supported by BRACE (Bristol Research into Alzheimer's and Care of the Elderly), Brains for Dementia Research and the Medical Research Council. The Neuropathology Section, Department of Cellular Pathology, University Hospital Southampton NHS Foundation Trust, the Histochemistry Research Unit, and the Biomedical Imaging Unit of the Faculty of Medicine, University of Southampton facilitated tissue processing, staining and analysis. Staff at Elan Pharmaceuticals made available original clinical trial data.

References

1. Amin J, Paquet C, Baker A, Asuni AA, Love S, Holmes C, Hugon J, Nicoll JA, Boche D (2014) Effect of Abeta immunisation on hyperphosphorylated tau: a potential role for GSK-3beta. Neuropathol Appl Neurobiol.
2. Ayala-Grosso C, Tam J, Roy S, Xanthoudakis S, Da Costa D, Nicholson DW, Robertson GS (2006) Caspase-3 cleaved spectrin colocalizes with neurofilament-immunoreactive neurons in Alzheimer's disease. Neuroscience.141(2):863-74.
3. Barnett A, Brewer GJ (2011) Autophagy in aging and Alzheimer's disease: pathologic or protective? J Alzheimers Dis.25(3):385-94.
4. Boche D, Donald J, Love S, Harris S, Neal JW, Holmes C, Nicoll JA (2010) Reduction of aggregated Tau in neuronal processes but not in the cell bodies after Abeta42 immunisation in Alzheimer's disease. Acta Neuropathol.120(1):13-20.
5. Boche D, Zotova E, Weller RO, Love S, Neal JW, Pickering RM, Wilkinson D, Holmes C, Nicoll JA (2008) Consequence of Abeta immunization on the vasculature of human Alzheimer's disease brain. Brain.131(Pt 12):3299-310.
6. Bose A, Mouton-Liger F, Paquet C, Mazot P, Vigny M, Gray F, Hugon J (2011) Modulation of tau phosphorylation by the kinase PKR: implications in Alzheimer's disease. Brain Pathol.21(2):189-200.
7. Chang RC, Suen KC, Ma CH, Elyaman W, Ng HK, Hugon J (2002) Involvement of double-stranded RNA-dependent protein kinase and phosphorylation of eukaryotic initiation factor-2alpha in neuronal degeneration. Journal of neurochemistry.83(5):1215-25.
8. Checler F, Alves da Costa C (2014) p53 in neurodegenerative diseases and brain cancers. Pharmacol Ther.142(1):99-113.
9. Dhanasekaran DN, Reddy EP (2008) JNK signaling in apoptosis. Oncogene.27(48):6245-51.
10. Dumurgier J, Mouton-Liger F, Lapalus P, Prevot M, Laplanche JL, Hugon J, Paquet C, Groupe d'Investigation du Liquide Cephalorachidien Study N (2013) Cerebrospinal fluid PKR level predicts cognitive decline in Alzheimer's disease. PloS one.8(1):e53587.
11. Elmore S (2007) Apoptosis: a review of programmed cell death. Toxicol Pathol.35(4):495-516.
12. Engmann O, Giese KP (2009) Crosstalk between Cdk5 and GSK3beta: Implications for Alzheimer's Disease. Front Mol Neurosci.2:2.
13. Flaherty DB, Soria JP, Tomasiewicz HG, Wood JG (2000) Phosphorylation of human tau protein by microtubule-associated kinases: GSK3beta and cdk5 are key participants. J Neurosci Res.62(3):463-72.
14. Frake RA, Ricketts T, Menzies FM, Rubinsztein DC (2015) Autophagy and neurodegeneration. J Clin Invest.125(1):65-74.
15. Friedlander RM (2003) Apoptosis and caspases in neurodegenerative diseases. N Engl J Med.348(14):1365-75.
16. Funderburk SF, Marcellino BK, Yue Z (2010) Cell "self-eating" (autophagy) mechanism in Alzheimer's disease. Mt Sinai J Med.77(1):59-68.
17. Gastard MC, Troncoso JC, Koliatsos VE (2003) Caspase activation in the limbic cortex of subjects with early Alzheimer's disease. Ann Neurol.54(3):393-8.
18. Gourmaud S, Paquet C, Dumurgier J, Pace C, Bouras C, Gray F, Laplanche JL, Meurs EF, Mouton-Liger F, Hugon J (2014) Increased levels of cerebrospinal fluid JNK3 associated with amyloid pathology: links to cognitive decline. J Psychiatry Neurosci.39(6):140062.
19. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA (2008) Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet.372(9634):216-23.
20. Hyman BT, Trojanowski JQ (1997) Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. J Neuropathol Exp Neurol.56(10):1095-7.

21. Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, Ahn HJ, Ait-Mohamed O, Ait-Si-Ali S, Akematsu T, Akira S, Al-Younes HM, Al-Zeer MA, Albert ML, Albin RL, Alegre-Abarrategui J, Aleo MF, Alirezaei M, Almasan A, Almonte-Becerril M, Amano A, Amaravadi R, Amarnath S, Amer AO, Andrieu-Abadie N, Anantharam V, Ann DK, Anoopkumar-Dukie S, Aoki H, Apostolova N, Arancia G, Aris JP, Asanuma K, Asare NY, Ashida H, Askanas V, Askew DS, Auberger P, Baba M, Backues SK, Baehrecke EH, Bahr BA, Bai XY, Bailly Y, Baiocchi R, Baldini G, Balduini W, Ballabio A, Bamber BA, Bampton ET, Banhegyi G, Bartholomew CR, Bassham DC, Bast RC, Jr., Batoko H, Bay BH, Beau I, Bechet DM, Begley TJ, Behl C, Behrends C, Bekri S, Bellaire B, Bendall LJ, Benetti L, Berliocchi L, Bernardi H, Bernassola F, Besteiro S, Bhatia-Kissova I, Bi X, Biard-Piechaczyk M, Blum JS, Boise LH, Bonaldo P, Boone DL, Bornhauser BC, Bortoluci KR, Bossis I, Bost F, Bourquin JP, Boya P, Boyer-Guittaut M, Bozhkov PV, Brady NR, Brancolini C, Brech A, Brenman JE, Brennand A, Bresnick EH, Brest P, Bridges D, Bristol ML, Brookes PS, Brown EJ, Brumell JH, Brunetti-Pierri N, Brunk UT, Bulman DE, Bultman SJ, Bultynck G, Burbulla LF, Bursch W, Butchar JP, Buzgariu W, Bydlowski SP, Cadwell K, Cahova M, Cai D, Cai J, Cai Q, Calabretta B, Calvo-Garrido J, Camougrand N, Campanella M, Campos-Salinas J, Candi E, Cao L, Caplan AB, Carding SR, Cardoso SM, Carew JS, Carlin CR, Carmignac V, Carneiro LA, Carra S, Caruso RA, Casari G, Casas C, Castino R, Cebollero E, Cecconi F, Celli J, Chaachouay H, Chae HJ, Chai CY, Chan DC, Chan EY, Chang RC, Che CM, Chen CC, Chen GC, Chen GQ, Chen M, Chen Q, Chen SS, Chen W, Chen X, Chen X, Chen X, Chen YG, Chen Y, Chen Y, Chen YJ, Chen Z, Cheng A, Cheng CH, Cheng Y, Cheong H, Cheong JH, Cherry S, Chess-Williams R, Cheung ZH, Chevet E, Chiang HL, Chiarelli R, Chiba T, Chin LS, Chiou SH, Chisari FV, Cho CH, Cho DH, Choi AM, Choi D, Choi KS, Choi ME, Chouaib S, Choubey D, Choubey V, Chu CT, Chuang TH, Chueh SH, Chun T, Chwae YJ, Chye ML, Ciarcia R, Ciriolo MR, Clague MJ, Clark RS, Clarke PG, Clarke R, Codogno P, Coller HA, Colombo MI, Comincini S, Condello M, Condorelli F, Cookson MR, Coombs GH, Coppens I, Corbalan R, Cossart P, Costelli P, Costes S, Coto-Montes A, Couve E, Coxon FP, Cregg JM, Crespo JL, Cronje MJ, Cuervo AM, Cullen JJ, Czaja MJ, D'Amelio M, Darfeuille-Michaud A, Davids LM, Davies FE, De Felici M, de Groot JF, de Haan CA, De Martino L, De Milito A, De Tata V, Debnath J, Degterev A, Dehay B, Delbridge LM, Demarchi F, Deng YZ, Dengjel J, Dent P, Denton D, Deretic V, Desai SD, Devenish RJ, Di Gioacchino M, Di Paolo G, Di Pietro C, Diaz-Araya G, Diaz-Laviada I, Diaz-Meco MT, Diaz-Nido J, Dikic I, Dinesh-Kumar SP, Ding WX, Distelhorst CW, Diwan A, Djavaheri-Mergny M, Dokudovskaya S, Dong Z, Dorsey FC, Dosenko V, Dowling JJ, Doxsey S, Dreux M, Drew ME, Duan Q, Duchosal MA, Duff K, Dugail I, Durbeej M, Duszenko M, Edelstein CL, Edinger AL, Egea G, Eichinger L, Eissa NT, Ekmekcioglu S, El-Deiry WS, Elazar Z, Elgendy M, Ellerby LM, Eng KE, Engelbrecht AM, Engelender S, Erenpreisa J, Escalante R, Esclatine A, Eskelinen EL, Espert L, Espina V, Fan H, Fan J, Fan QW, Fan Z, Fang S, Fang Y, Fanto M, Fanzani A, Farkas T, Farre JC, Faure M, Fechheimer M, Feng CG, Feng J, Feng Q, Feng Y, Fesus L, Feuer R, Figueiredo-Pereira ME, Fimia GM, Fingar DC, Finkbeiner S, Finkel T, Finley KD, Fiorito F, Fisher EA, Fisher PB, Flajolet M, Florez-McClure ML, Florio S, Fon EA, Fornai F, Fortunato F, Fotedar R, Fowler DH, Fox HS, Franco R, Frankel LB, Fransen M, Fuentes JM, Fueyo J, Fujii J, Fujisaki K, Fujita E, Fukuda M, Furukawa RH, Gaestel M, Gailly P, Gajewska M, Galliot B, Galy V, Ganesh S, Ganetzky B, Ganley IG, Gao FB, Gao GF, Gao J, Garcia L, Garcia-Manero G, Garcia-Marcos M, Garmyn M, Gartel AL, Gatti E, Gautel M, Gawriluk TR, Gegg ME, Geng J, Germain M, Gestwicki JE, Gewirtz DA, Ghavami S, Ghosh P, Giammarioli AM, Giatromanolaki AN, Gibson SB, Gilkerson RW, Ginger ML, Ginsberg HN, Golab J, Goligorsky MS, Golstein P, Gomez-Manzano C, Goncu E, Gongora C, Gonzalez CD, Gonzalez R, Gonzalez-Estevez C, Gonzalez-Polo RA, Gonzalez-Rey E, Gorbunov NV, Gorski S, Goruppi S, Gottlieb RA, Gozuacik D, Granato GE, Grant GD, Green KN, Gregorc A, Gros F, Grose C, Grunt TW, Gual P, Guan JL, Guan KL, Guichard SM, Gukovskaya AS, Gukovsky I, Gunst J, Gustafsson AB, Halayko AJ, Hale AN, Halonen SK, Hamasaki M, Han F, Han T, Hancock MK, Hansen M, Harada H, Harada M, Hardt SE, Harper JW, Harris AL, Harris J, Harris SD, Hashimoto M, Haspel JA, Hayashi S, Hazelhurst LA, He C, He YW, Hebert MJ, Heidenreich KA, Helfrich MH, Helgason GV, Henske EP, Herman B, Herman PK, Hetz C, Hilfiker S, Hill JA, Hocking LJ, Hofman P, Hofmann TG, Hohfeld J, Holyoake TL, Hong MH, Hood DA, Hotamisligil GS, Houwerzijl EJ, Hoyer-Hansen M, Hu B, Hu CA, Hu HM, Hua Y, Huang C, Huang J, Huang S, Huang WP, Huber TB, Huh WK, Hung TH, Hupp TR, Hur GM, Hurley JB, Hussain SN, Hussey PJ, Hwang

JJ, Hwang S, Ichihara A, Ilkhanizadeh S, Inoki K, Into T, Iovane V, Iovanna JL, Ip NY, Isaka Y, Ishida H, Isidoro C, Isobe K, Iwasaki A, Izquierdo M, Izumi Y, Jaakkola PM, Jaattela M, Jackson GR, Jackson WT, Janji B, Jendrach M, Jeon JH, Jeung EB, Jiang H, Jiang H, Jiang JX, Jiang M, Jiang Q, Jiang X, Jiang X, Jimenez A, Jin M, Jin S, Joe CO, Johansen T, Johnson DE, Johnson GV, Jones NL, Joseph B, Joseph SK, Joubert AM, Juhasz G, Juillerat-Jeanneret L, Jung CH, Jung YK, Kaarniranta K, Kaasik A, Kabuta T, Kadowaki M, Kagedal K, Kamada Y, Kaminskyy VO, Kampinga HH, Kanamori H, Kang C, Kang KB, Kang KI, Kang R, Kang YA, Kanki T, Kanneganti TD, Kanno H, Kanthasamy AG, Kanthasamy A, Karantza V, Kaushal GP, Kaushik S, Kawazoe Y, Ke PY, Kehrl JH, Kelekar A, Kerkhoff C, Kessel DH, Khalil H, Kiel JA, Kiger AA, Kihara A, Kim DR, Kim DH, Kim DH, Kim EK, Kim HR, Kim JS, Kim JH, Kim JC, Kim JK, Kim PK, Kim SW, Kim YS, Kim Y, Kimchi A, Kimmelman AC, King JS, Kinsella TJ, Kirkin V, Kirshenbaum LA, Kitamoto K, Kitazato K, Klein L, Klimecki WT, Klucken J, Knecht E, Ko BC, Koch JC, Koga H, Koh JY, Koh YH, Koike M, Komatsu M, Kominami E, Kong HJ, Kong WJ, Korolchuk VI, Kotake Y, Koukourakis MI, Kouri Flores JB, Kovacs AL, Kraft C, Krainc D, Kramer H, Kretz-Remy C, Krichevsky AM, Kroemer G, Kruger R, Krut O, Ktistakis NT, Kuan CY, Kucharczyk R, Kumar A, Kumar R, Kumar S, Kundu M, Kung HJ, Kurz T, Kwon HJ, La Spada AR, Lafont F, Lamark T, Landry J, Lane JD, Lapaquette P, Laporte JF, Laszlo L, Lavandero S, Lavoie JN, Layfield R, Lazo PA, Le W, Le Cam L, Ledbetter DJ, Lee AJ, Lee BW, Lee GM, Lee J, Lee JH, Lee M, Lee MS, Lee SH, Leeuwenburgh C, Legembre P, Legouis R, Lehmann M, Lei HY, Lei QY, Leib DA, Leiro J, Lemasters JJ, Lemoine A, Lesniak MS, Lev D, Levenson VV, Levine B, Levy E, Li F, Li JL, Li L, Li S, Li W, Li XJ, Li YB, Li YP, Liang C, Liang Q, Liao YF, Liberski PP, Lieberman A, Lim HJ, Lim KL, Lim K, Lin CF, Lin FC, Lin J, Lin JD, Lin K, Lin WW, Lin WC, Lin YL, Linden R, Lingor P, Lippincott-Schwartz J, Lisanti MP, Liton PB, Liu B, Liu CF, Liu K, Liu L, Liu QA, Liu W, Liu YC, Liu Y, Lockshin RA, Lok CN, Lonial S, Loos B, Lopez-Berestein G, Lopez-Otin C, Lossi L, Lotze MT, Low P, Lu B, Lu B, Lu B, Lu Z, Luciano F, Lukacs NW, Lund AH, Lynch-Day MA, Ma Y, Macian F, MacKeigan JP, Macleod KF, Madeo F, Maiuri L, Maiuri MC, Malagoli D, Malicdan MC, Malorni W, Man N, Mandelkow EM, Manon S, Manov I, Mao K, Mao X, Mao Z, Marambaud P, Marazziti D, Marcel YL, Marchbank K, Marchetti P, Marciniak SJ, Marcondes M, Mardi M, Marfe G, Marino G, Markaki M, Marten MR, Martin SJ, Martinand-Mari C, Martinet W, Martinez-Vicente M, Masini M, Matarrese P, Matsuo S, Matteoni R, Mayer A, Mazure NM, McConkey DJ, McConnell MJ, McDermott C, McDonald C, McInerney GM, McKenna SL, McLaughlin B, McLean PJ, McMaster CR, McQuibban GA, Meijer AJ, Meisler MH, Melendez A, Melia TJ, Melino G, Mena MA, Menendez JA, Menna-Barreto RF, Menon MB, Menzies FM, Mercer CA, Merighi A, Merry DE, Meschini S, Meyer CG, Meyer TF, Miao CY, Miao JY, Michels PA, Michiels C, Mijaljica D, Milojkovic A, Minucci S, Miracco C, Miranti CK, Mitroulis I, Miyazawa K, Mizushima N, Mograbi B, Mohseni S, Molero X, Mollereau B, Mollinedo F, Momoi T, Monastyrska I, Monick MM, Monteiro MJ, Moore MN, Mora R, Moreau K, Moreira PI, Moriyasu Y, Moscat J, Mostowy S, Mottram JC, Motyl T, Moussa CE, Muller S, Muller S, Munger K, Munz C, Murphy LO, Murphy ME, Musaro A, Mysorekar I, Nagata E, Nagata K, Nahimana A, Nair U, Nakagawa T, Nakahira K, Nakano H, Nakatogawa H, Nanjundan M, Naqvi NI, Narendra DP, Narita M, Navarro M, Nawrocki ST, Nazarko TY, Nemchenko A, Netea MG, Neufeld TP, Ney PA, Nezis IP, Nguyen HP, Nie D, Nishino I, Nislow C, Nixon RA, Noda T, Noegel AA, Nogalska A, Noguchi S, Notterpek L, Novak I, Nozaki T, Nukina N, Nurnberger T, Nyfeler B, Obara K, Oberley TD, Oddo S, Ogawa M, Ohashi T, Okamoto K, Oleinick NL, Oliver FJ, Olsen LJ, Olsson S, Opota O, Osborne TF, Ostrander GK, Otsu K, Ou JH, Ouimet M, Overholtzer M, Ozpolat B, Paganetti P, Pagnini U, Pallet N, Palmer GE, Palumbo C, Pan T, Panaretakis T, Pandey UB, Papackova Z, Papassideri I, Paris I, Park J, Park OK, Parys JB, Parzych KR, Patschan S, Patterson C, Pattingre S, Pawelek JM, Peng J, Perlmutter DH, Perrotta I, Perry G, Pervaiz S, Peter M, Peters GJ, Petersen M, Petrovski G, Phang JM, Piacentini M, Pierre P, Pierrefite-Carle V, Pierron G, Pinkas-Kramarski R, Piras A, Piri N, Platanias LC, Poggeler S, Poirot M, Poletti A, Pous C, Pozuelo-Rubio M, Praetorius-Ibba M, Prasad A, Prescott M, Priault M, Produit-Zengaffinen N, Progulske-Fox A, Proikas-Cezanne T, Przedborski S, Przyklenk K, Puertollano R, Puyal J, Qian SB, Qin L, Qin ZH, Quaggin SE, Raben N, Rabinowich H, Rabkin SW, Rahman I, Rami A, Ramm G, Randall G, Randow F, Rao VA, Rathmell JC, Ravikumar B, Ray SK, Reed BH, Reed JC, Reggiori F, Regnier-Vigouroux A, Reichert AS, Reiners JJ, Jr., Reiter RJ, Ren J, Revuelta JL, Rhodes CJ, Ritis K, Rizzo E, Robbins J, Roberge M, Roca H, Roccheri MC, Rocchi S, Rodemann HP, Rodriguez de Cordoba S, Rohrer B, Roninson IB, Rosen K, Rost-

Roszkowska MM, Rouis M, Rouschop KM, Rovetta F, Rubin BP, Rubinsztein DC, Ruckdeschel K, Rucker EB, 3rd, Rudich A, Rudolf E, Ruiz-Opazo N, Russo R, Rusten TE, Ryan KM, Ryter SW, Sabatini DM, Sadoshima J, Saha T, Saitoh T, Sakagami H, Sakai Y, Salekdeh GH, Salomoni P, Salvaterra PM, Salvesen G, Salvioli R, Sanchez AM, Sanchez-Alcazar JA, Sanchez-Prieto R, Sandri M, Sankar U, Sansanwal P, Santambrogio L, Saran S, Sarkar S, Sarwal M, Sasakawa C, Sasnauskiene A, Sass M, Sato K, Sato M, Schapira AH, Scharl M, Schatzl HM, Scheper W, Schiaffino S, Schneider C, Schneider ME, Schneider-Stock R, Schoenlein PV, Schorderet DF, Schuller C, Schwartz GK, Scorrano L, Sealy L, Seglen PO, Segura-Aguilar J, Seiliez I, Seleverstov O, Sell C, Seo JB, Separovic D, Setaluri V, Setoguchi T, Settembre C, Shacka JJ, Shanmugam M, Shapiro IM, Shaulian E, Shaw RJ, Shelhamer JH, Shen HM, Shen WC, Sheng ZH, Shi Y, Shibuya K, Shidoji Y, Shieh JJ, Shih CM, Shimada Y, Shimizu S, Shintani T, Shirihai OS, Shore GC, Sibirny AA, Sidhu SB, Sikorska B, Silva-Zacarin EC, Simmons A, Simon AK, Simon HU, Simone C, Simonsen A, Sinclair DA, Singh R, Sinha D, Sinicrope FA, Sirko A, Siu PM, Sivridis E, Skop V, Skulachev VP, Slack RS, Smaili SS, Smith DR, Soengas MS, Soldati T, Song X, Sood AK, Soong TW, Sotgia F, Spector SA, Spies CD, Springer W, Srinivasula SM, Stefanis L, Steffan JS, Stendel R, Stenmark H, Stephanou A, Stern ST, Sternberg C, Stork B, Stralfors P, Subauste CS, Sui X, Sulzer D, Sun J, Sun SY, Sun ZJ, Sung JJ, Suzuki K, Suzuki T, Swanson MS, Swanton C, Sweeney ST, Sy LK, Szabadkai G, Tabas I, Taegtmeyer H, Tafani M, Takacs-Vellai K, Takano Y, Takegawa K, Takemura G, Takeshita F, Talbot NJ, Tan KS, Tanaka K, Tanaka K, Tang D, Tang D, Tanida I, Tannous BA, Tavernarakis N, Taylor GS, Taylor GA, Taylor JP, Terada LS, Terman A, Tettamanti G, Thevissen K, Thompson CB, Thorburn A, Thumm M, Tian F, Tian Y, Tocchini-Valentini G, Tolkovsky AM, Tomino Y, Tonges L, Tooze SA, Tournier C, Tower J, Towns R, Trajkovic V, Travassos LH, Tsai TF, Tschan MP, Tsubata T, Tsung A, Turk B, Turner LS, Tyagi SC, Uchiyama Y, Ueno T, Umekawa M, Umemiya-Shirafuji R, Unni VK, Vaccaro MI, Valente EM, Van den Berghe G, van der Klei IJ, van Doorn W, van Dyk LF, van Egmond M, van Grunsven LA, Vandenabeele P, Vandenberghe WP, Vanhorebeek I, Vaquero EC, Velasco G, Vellai T, Vicencio JM, Vierstra RD, Vila M, Vindis C, Viola G, Viscomi MT, Voitsekhovskaja OV, von Haefen C, Votruba M, Wada K, Wade-Martins R, Walker CL, Walsh CM, Walter J, Wan XB, Wang A, Wang C, Wang D, Wang F, Wang F, Wang G, Wang H, Wang HG, Wang HD, Wang J, Wang K, Wang M, Wang RC, Wang X, Wang X, Wang YJ, Wang Y, Wang Z, Wang ZC, Wang Z, Wansink DG, Ward DM, Watada H, Waters SL, Webster P, Wei L, Weihl CC, Weiss WA, Welford SM, Wen LP, Whitehouse CA, Whitton JL, Whitworth AJ, Wileman T, Wiley JW, Wilkinson S, Willbold D, Williams RL, Williamson PR, Wouters BG, Wu C, Wu DC, Wu WK, Wyttenbach A, Xavier RJ, Xi Z, Xia P, Xiao G, Xie Z, Xie Z, Xu DZ, Xu J, Xu L, Xu X, Yamamoto A, Yamamoto A, Yamashina S, Yamashita M, Yan X, Yanagida M, Yang DS, Yang E, Yang JM, Yang SY, Yang W, Yang WY, Yang Z, Yao MC, Yao TP, Yeganeh B, Yen WL, Yin JJ, Yin XM, Yoo OJ, Yoon G, Yoon SY, Yorimitsu T, Yoshikawa Y, Yoshimori T, Yoshimoto K, You HJ, Youle RJ, Younes A, Yu L, Yu L, Yu SW, Yu WH, Yuan ZM, Yue Z, Yun CH, Yuzaki M, Zabirnyk O, Silva-Zacarin E, Zacks D, Zacksenhaus E, Zaffaroni N, Zakeri Z, Zeh HJ, 3rd, Zeitlin SO, Zhang H, Zhang HL, Zhang J, Zhang JP, Zhang L, Zhang L, Zhang MY, Zhang XD, Zhao M, Zhao YF, Zhao Y, Zhao ZJ, Zheng X, Zhivotovsky B, Zhong Q, Zhou CZ, Zhu C, Zhu WG, Zhu XF, Zhu X, Zhu Y, Zoladek T, Zong WX, Zorzano A, Zschocke J, Zuckerbraun B (2012) Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy.8(4):445-544.

22. Klionsky DJ, Emr SD (2000) Autophagy as a regulated pathway of cellular degradation. Science.290(5497):1717-21.

23. Lee MS, Tsai LH (2003) Cdk5: one of the links between senile plaques and neurofibrillary tangles? J Alzheimers Dis.5(2):127-37.

24. Li Q, Liu Y, Sun M (2016) Autophagy and Alzheimer's Disease. Cell Mol Neurobiol.

25. Ma Y, Bao J, Zhao X, Shen H, Lv J, Ma S, Zhang X, Li Z, Wang S, Wang Q, Ji J (2013) Activated cyclin-dependent kinase 5 promotes microglial phagocytosis of fibrillar beta-amyloid by up-regulating lipoprotein lipase expression. Mol Cell Proteomics.12(10):2833-44.

26. Marino G, Niso-Santano M, Baehrecke EH, Kroemer G (2014) Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol.15(2):81-94.

27. Mattson MP (2000) Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol.1(2):120-9.

28. Mizushima N, Yoshimori T, Levine B (2010) Methods in mammalian autophagy research. Cell.140(3):313-26.
29. Mouton-Liger F, Paquet C, Dumurgier J, Bouras C, Pradier L, Gray F, Hugon J (2012) Oxidative stress increases BACE1 protein levels through activation of the PKR-eIF2alpha pathway. Biochimica et biophysica acta.1822(6):885-96.
30. Nicoll JA, Barton E, Boche D, Neal JW, Ferrer I, Thompson P, Vlachouli C, Wilkinson D, Bayer A, Games D, Seubert P, Schenk D, Holmes C (2006) Abeta species removal after abeta42 immunization. J Neuropathol Exp Neurol.65(11):1040-8.
31. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med.9(4):448-52.
32. Obulesu M, Lakshmi MJ (2014) Apoptosis in Alzheimer's disease: an understanding of the physiology, pathology and therapeutic avenues. Neurochem Res.39(12):2301-12.
33. Ogolla PS, Portillo JA, White CL, Patel K, Lamb B, Sen GC, Subauste CS (2013) The protein kinase double-stranded RNA-dependent (PKR) enhances protection against disease cause by a non-viral pathogen. PLoS Pathog.9(8):e1003557.
34. Paquet C, Amin J, Mouton-Liger F, Nasser M, Love S, Gray F, Pickering RM, Nicoll JA, Holmes C, Hugon J, Boche D (2014) Effect of active Abeta immunotherapy on neurons in human Alzheimer's disease. J Pathol.
35. Pareek TK, Lam E, Zheng X, Askew D, Kulkarni AB, Chance MR, Huang AY, Cooke KR, Letterio JJ (2010) Cyclin-dependent kinase 5 activity is required for T cell activation and induction of experimental autoimmune encephalomyelitis. J Exp Med.207(11):2507-19.
36. Peel AL (2004) PKR activation in neurodegenerative disease. Journal of neuropathology and experimental neurology.63(2):97-105.
37. Pei JJ, Braak E, Braak H, Grundke-Iqbal I, Iqbal K, Winblad B, Cowburn RF (1999) Distribution of active glycogen synthase kinase 3beta (GSK-3beta) in brains staged for Alzheimer disease neurofibrillary changes. J Neuropathol Exp Neurol.58(9):1010-9.
38. Rubinsztein DC, Shpilka T, Elazar Z (2012) Mechanisms of autophagosome biogenesis. Curr Biol.22(1):R29-34.
39. Serrano-Pozo A, William CM, Ferrer I, Uro-Coste E, Delisle MB, Maurage CA, Hock C, Nitsch RM, Masliah E, Growdon JH, Frosch MP, Hyman BT (2010) Beneficial effect of human anti-amyloid-beta active immunization on neurite morphology and tau pathology. Brain.133(Pt 5):1312-27.
40. Stadelmann C, Deckwerth TL, Srinivasan A, Bancher C, Bruck W, Jellinger K, Lassmann H (1999) Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death. Am J Pathol.155(5):1459-66.
41. Su JH, Zhao M, Anderson AJ, Srinivasan A, Cotman CW (2001) Activated caspase-3 expression in Alzheimer's and aged control brain: correlation with Alzheimer pathology. Brain Res.898(2):350-7.
42. Sun KH, Lee HG, Smith MA, Shah K (2009) Direct and indirect roles of cyclin-dependent kinase 5 as an upstream regulator in the c-Jun NH2-terminal kinase cascade: relevance to neurotoxic insults in Alzheimer's disease. Mol Biol Cell.20(21):4611-9.
43. Yarza R, Vela S, Solas M, Ramirez MJ (2015) c-Jun N-terminal Kinase (JNK) Signaling as a Therapeutic Target for Alzheimer's Disease. Front Pharmacol.6:321.
44. Zare-Shahabadi A, Masliah E, Johnson GV, Rezaei N (2015) Autophagy in Alzheimer's disease. Rev Neurosci.26(4):385-95.
45. Zhu X, Raina AK, Rottkamp CA, Aliev G, Perry G, Boux H, Smith MA (2001) Activation and redistribution of c-jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease. J Neurochem.76(2):435-41.
46. Zotova E, Bharambe V, Cheaveau M, Morgan W, Holmes C, Harris S, Neal JW, Love S, Nicoll JA, Boche D (2013) Inflammatory components in human Alzheimer's disease and after active amyloid-beta42 immunization. Brain.136(Pt 9):2677-96.

Table 1 Characteristics of the immunised (iAD) and non-immunised (cAD) Alzheimer's disease cohorts

ID case	Gender	Age	Braak stage	Dementia duration (years)	APOE status	Mean antibody response (ELISA units)	Survival time from 1st injection (months)	*Post-mortem* delay (hours)
iAD1	F	74	VI	6	3.4	1:119	20	48
iAD2	M	83	V	11	3.3	<1:100	4	6
iAD3	M	63	VI	6	3.3	<1:100	41	6
iAD4	F	71	VI	10	3.3	1:4072	44	24
iAD5	M	81	VI	7	3.4	1:1707	57	6
iAD6	M	82	VI	6	3.4	1:4374	60	24
iAD7	M	63	VI	10	3.4	1:6470	64	6
iAD8	M	81	VI	11	4.4	1:491	63	?
iAD9	F	88	VI	11	3.3	1:137	86	24
iAD10	M	88	VI	12	3.4	1:142	94	6
iAD11	F	89	VI	15	3.4	1:142	111	?
iAD12	F	86	VI	13	4.4	<1:100	141	6
iAD13	F	75	VI	19	?	1:221	162	48
cAD (*n*=28)	15F:13M	63-88	V/VI	3-17	21$\varepsilon4^{+}$:6 $\varepsilon4^{-}$	n/a	n/a	mean 39 median 26

n/a: non-applicable

?: unknown

Table 2: Topographical distribution of the apoptotic and autophagic proteins.

cAD	**Neurons**		**Glial cells**	
	Cytoplasm	Nuclear	Cytoplasm	Nuclear
a-casp3	+	-	+	-
Cdk5/p35	+	-	+	-
pJNK	+	-	+	-
$GSK3\beta_{tyr216}$	+	+	-	-
P53	+	-	-	-
LC3	+	-	+	-
ATG5	+	-	-	+

iAD	**Neurons**		**Glial cells**	
	Cytoplasm	Nuclear	Cytoplasm	Nuclear
a-casp3	+	-	-	-
Cdk5/p35	+	-	+	-
pJNK	+	-	-	-
$GSK3\beta_{tyr216}$	+	+	-	-
P53	+	-	-	-
LC3	+	-	-	-
ATG5	+	-	-	+

Table 3: Results of correlation analyses within the non-immunized AD control group

	pJNK	Cdk5/p35	p53	a-casp3	GSK3β$_{tyr216}$	ATG5	LC3-II
Aβ42	r=0.141 p=0.483	r=-0.238 p=0.232	r=0.142 p=0.497	**r=0.561**** **p=0.005**	r=-0.079 p=0.696	r=-0.173 p=0.399	r=-0.346 p=0.090
ptau	r=-0.228 p=0.252	r=0.178 p=0.374	r=0.052 p=0.804	r=-0.224 p=0.303	r=0.365 p=0.061	r=-0.214 p=0.295	r=0.060 p=0.777
tangles	r=-0.088 p=0.662	r=0.092 p=0.648	r=-0.254 p=0.221	r=-0.070 p=0.750	r=0.008 p=0.970	r=-0.387 p=0.050	r=-0.046 p=0.828
dystrophic neurites	r=0.157 p=0.433	r=0.001 p=0.998	r=0.094 p=0.655	r=0.068 p=0.758	r=-0.010 p=0.959	r=-0.235 p=0.248	r=0.027 p=0.898
spongiosis	r=-0.181 p=0.365	r=0.404 p=0.037	r=0.048 p=0.818	r=-0.327 p=0.128	r=0.166 p=0.409	r=0.231 p=0.256	r=0.084 p=0.690
NeuN	r=0.008 p=0.971	r=-0.039 p=0.860	r=0.413 p=0.063	r=-0.118 p=0.610	r=0.361 p=0.090	r=0.232 p=0.298	r=0.160 p=0.489
NFP curvature ratio	r=-0.042 p=0.837	r=0.180 p=0.369	r=0.182 p=0.383	r=-0.059 p=0.790	r=0.174 p=0.384	r=-0.055 p=0.788	r=0.134 p=0.524
pPKR	r=-0.267 p=0.178	r=0.085 p=0.673	r=-0.081 p=0.701	r=0.094 p=0.670	r=0.337 p=0.085	r=0.110 p=0.593	r=-0.075 p=0.723
pJNK		r=0.426 p=0.027	r=0.055 p=0.792	r=0.177 p=0.419	r=0.311 p=0.115	r=-0.226 p=0.266	r=0.202 p=0.334
Cdk5/p35			r=0.277 p=0.18	r=-0.146 p=0.505	**r=0.648**** **p<0.001**	r=-0.196 p=0.338	r=0.300 p=0.144
p53				r=0.172 p=0.457	r=0.280 p=0.175	r=-0.055 p=0.795	r=0.319 p=0.120
a-casp3					r=-0.136 p=0.536	r=-0.492 p=0.020	r=-0.157 p=0.496
GSK3β$_{tyr216}$						r=-0.01 p=0.927	r=0.128 p=0.542
ATG5							r=-0.062 p=0.770
Age at death	r=0.210 p=0.294	r=0.289 p=0.144	**r=0.564**** **p=0.003**	r=0.389 p=0.0670	r=0.438 p=0.022	r=-0.287 p=0.156	r=0.220 p=0.291
Dementia duration	r=0.057 p=0.796	r=0.372 p=0.080	r=0.388 p=0.082	r=-0.062 p=0.795	r=-0.008 p=0.970	r=0.049 p=0.830	r=0.691 p=0.001
Peak antibody	r=0.033 p=0.914	**r=0.840**** **p<0.001**	r=-0.175 p=0.569	r=-0.431 p=0.142	r=-0.284 p=0.348	r=0.459 p=0.115	r=-0.386 p=0.193
Survival time	r=0.455 p=0.119	r=0.162 p=0.590	r=-0.077 p=0.802	r=0.252 p=0.406	r=0.446 p=0.126	r=0.280 p=0.354	r=0.568 p=0.043

Bold: ** correlation significant at the 0.01 level (2-tailed).

Table 4: Results of correlation analyses within the immunized AD control group

	pJNK	Cdk5/p35	p53	a-casp3	GSK3β$_{tyr216}$	ATG5	LC3-II
Aβ42	r=-0.237 p=0.482	r=-0.491 p=0.125	r=-0.361 p=0.276	r=0.413 p=0.207	r=0.324 p=0.331	**r=-0.845**** **p=0.001**	r=0.484 p=0.131
ptau	r=0.397 p=0.226	r=0.082 p=0.811	r=0.164 p=0.629	r=0.089 p=0.794	r=-0.231 p=0.494	r=0.036 p=0.915	r=-0.174 p=0.610
tangles	r=0.301 p=0.368	r=0.464 p=0.151	r=-0.050 p=0.883	r=-0.089 p=0.796	r=-0.207 p=0.541	r=0.155 p=0.650	r=-0.507 p=0.112
dystrophic neurites	r=0.037 p=0.915	r=-0.246 p=0.466	r=-0.165 p=0.628	r=0.667 p=0.025	r=0.654 p=0.029	r=-0.269 p=0.424	r=0.547 p=0.082
spongiosis	r=0.479 p=0.136	r=0.009 p=0.979	r=-0.087 p=0.800	**r=0.789**** **p=0.004**	**r=0.761**** **p=0.007**	r=-0.055 p=0.873	r=0.128 p=0.708
NeuN	r=0.662 p=0.037	r=-0.353 p=0.318	r=0.107 p=0.769	r=0.691 p=0.027	r=0.337 p=0.340	r=0.170 p=0.638	r=0.055 p=0.880
NFP curvature ratio	r=0.448 p=0.167	r=0.377 p=0.253	r=0.194 p=0.568	r=-0.152 p=0.656	r=0.137 p=0.687	**r=0.841**** **p=0.001**	r=-0.418 p=0.201
pPKR	r=0.201 p=0.577	r=-0.564 p=0.090	r=0.213 p=0.555	r=0.297 p=0.405	r=0.258 p=0.471	r=-0.176 p=0.627	r=0.701 p=0.024
pJNK		r=0.11 p=0.720	r=0.083 p=0.788	r=0.534 p=0.060	r=0.078 p=0.801	r=0.529 0 p=.063	r=-0.300 p=0.319
Cdk5/p35			r=-0.223 p=0.464	r=-0.049 p=0.873	r=0.102 p=0.739	r=0.363 p=0.223	r=-0.342 p=0.253
p53				r=0.052 p=0.865	r=-0.233 p=0.444	r=0.165 p=0.589	r=0.268 p=0.375
a-casp3					r=0.546 p=0.054	r=-0.165 p=0.590	r=-0.069 p=0.823
GSK3β$_{tyr216}$						r=-0.108 p=0.726	r=-0.218 p=0.474
ATG5							r=-0.303 p=0.314
Age at death	r=0.512 p=0.074	r=-0.502 p=0.08	r=-0.029 p=0.925	r=-0.080 p=0.795	r=0.082 p=0.791	r=0.337 p=0.261	r=-0.262 p=0.388
Dementia duration	r=0.119 p=0.700	r=-0.125 p=0.684	r=-0.297 p=0.324	r=0.134 p=0.661	r=0.178 p=0.560	r=0.008 p=0.978	r=-0.292 p=0.333
Peak antibody	r=0.033 p=0.914	**r=0.840**** **p<0.001**	r=-0.175 p=0.569	r=-0.431 p=0.142	r=-0.284 p=0.348	r=0.459 p=0.115	r=-0.386 p=0.193
Survival time	r=0.455 p=0.119	r=0.162 p=0.590	r=-0.077 p=0.802	r=0.252 p=0.406	r=0.446 p=0.126	r=0.280 p=0.354	r=0.568 p=0.043

Bold: ** correlation significant at the 0.01 level (2-tailed).

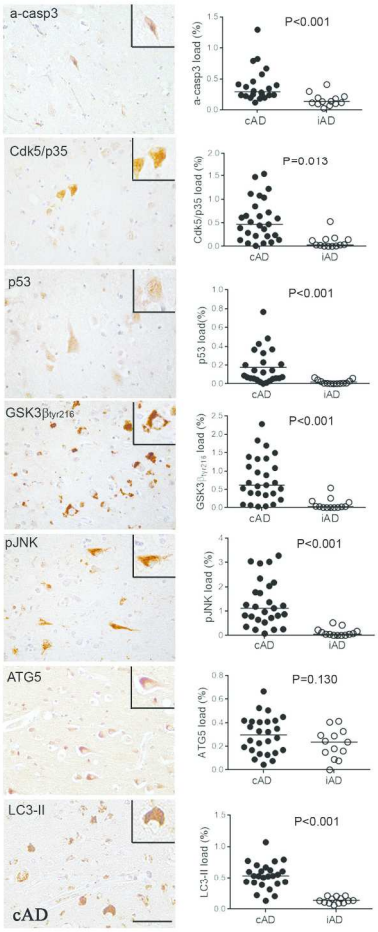

Figure 1: On the left, illustration of the immunolabeling of pro-apoptotic and autophagic proteins as observed in Alzheimer's disease. On the right, quantification of the proteins in the non-immunised AD (cAD) compared to immunised AD (iAD) cases showing a significant decrease in all apoptotic proteins and of LC3II after immunisation. Scale bar = 50µm.

99x279mm (300 x 300 DPI)