BMJ Open Antiviral therapy: Valacyclovir Treatment of Alzheimer's Disease (VALAD) Trial: protocol for a randomised, double-blind, placebo-controlled, treatment trial

D P Devanand,^{1,2,3,4} Howard Andrews,^{1,2,5} William C Kreisl,^{3,4} Qolamreza Razlighi,^{3,4} Anne Gershon,⁶ Yaakov Stern,^{1,3,4} Akiva Mintz,⁷ Thomas Wisniewski,⁸ Edward Acosta,⁹ Julianna Pollina,^{1,2} Mariasofia Katsikoumbas,^{1,2} Karen L Bell,^{3,4} Gregory H Pelton,^{1,2} Deborah Deliyannides,^{1,2} K M Prasad,¹⁰ Edward D Huey^{1,2,3,4}

To cite: Devanand DP, Andrews H, Kreisl WC, *et al.* Antiviral therapy: Valacyclovir Treatment of Alzheimer's Disease (VALAD) Trial: protocol for a randomised, double-blind, placebo-controlled, treatment trial. *BMJ Open* 2020;**10**:e032112. doi:10.1136/bmjopen-2019-032112

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2019-032112>).

Received 04 June 2019
Revised 16 October 2019
Accepted 26 November 2019

© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr D P Devanand;
dpd3@cumc.columbia.edu

ABSTRACT

Introduction After infection, herpes simplex virus-1 (HSV1) becomes latent in the trigeminal ganglion and can enter the brain via retrograde axonal transport. Recurrent reactivation of HSV1 may lead to neurodegeneration and Alzheimer's disease (AD) pathology. HSV1 (oral herpes) and HSV2 (genital herpes) can trigger amyloid beta-protein (Aβ) aggregation and HSV1 DNA is common in amyloid plaques. Anti-HSV drugs reduce Aβ and phosphorylated tau accumulation in cell-culture models. Cognitive impairment is greater in patients with HSV seropositive, and antiviral drugs show robust efficacy against peripheral HSV infection. Recent studies of electronic health records databases demonstrate that HSV infections increase dementia risk, and that antiviral medication treatment reduces this risk. The generic antiviral drug valacyclovir was superior to placebo in improving memory in a schizophrenia pilot trial but has not been tested in AD.

Methods and analysis In patients with mild AD who test positive for HSV1 or HSV2 serum antibodies, valacyclovir, repurposed as an anti-AD drug, will be compared with placebo (lactose pills) in 130 patients (65 valacyclovir and 65 placebo) in a randomised, double-blind, 78-week phase II proof-of-concept trial. Patients on valacyclovir, dose-titrated from 2 g to a targeted oral dose of 4 g daily, compared with placebo, are hypothesised to show smaller cognitive and functional decline, and, using ¹⁸F-Florbetapir positron emission tomography (PET) and ¹⁸F-MK-6240 PET imaging, to show less amyloid and tau accumulation, respectively. In the lumbar puncture subsample, cerebrospinal fluid acyclovir will be assayed to assess central nervous system valacyclovir penetration.

Ethics and dissemination The trial is being overseen by the New York State Psychiatric Institute Institutional Review Board (protocol 7537), the National Institute on Ageing, and the Data Safety Monitoring Board. Written informed consent is obtained for all subjects. Results will be disseminated via publication, clinicaltrials.gov, media and conferences.

Trial registration number ClinicalTrials.gov identifier (NCT03282916) Pre-results.

Strengths and limitations of this study

- The association between herpes simplex virus-1 (HSV1) and cognitive impairment meets several Bradford-Hill criteria suggesting a cause and effect relationship with consistent evidence of impairment, modest effect size, and a temporal relationship between HSV exposure and cognitive deficits.
- First randomised, double-blind, placebo-controlled trial of an antiviral treatment in Alzheimer's disease (AD) or any dementia that evaluates clinically relevant cognitive and functional outcomes.
- Evaluation of the impact of antiviral treatment on biomarkers: positron emission tomography amyloid and tau imaging indices, and secondary measures of MRI cortical thinning, anti-HSV antibodies and odour identification impairment.
- A linear association between virus exposure dose and illness severity cannot be tested accurately because the frequency of seropositivity is high in older adults and antibody levels reflect both old and new infections.
- HSV is unlikely to be the sole cause of AD as some individuals with HSV seropositive do not develop AD and individuals with HSV seronegative can develop AD.

INTRODUCTION

Some viruses can cause neurodegenerative disorders, for example, measles virus infection can lead, years later, to subacute sclerosing panencephalitis.¹ Alzheimer's disease (AD) may be transmissible in mice and primates, possibly by a virus.^{2 3} The long-standing viral aetiology hypothesis of AD posits that viruses in the brain, primarily herpes simplex virus-1 (HSV1) (causes oral herpes) and possibly HSV2 (causes genital herpes), may be aetiological or contribute to

the pathology of AD.^{4 5} There is growing scientific recognition that microbes, particularly viruses like HSV1, may be a cause of AD or contribute to its pathology, and that an antiviral treatment trial is needed.¹⁶ An emerging view is that amyloid may be a consequence of infection, and may have protective effects.⁷

Effects of HSV on AD neuropathology

In experimental studies, HSV1 infection of neuronal and glial cells triggers a decrease in amyloid precursor protein, an increase in intracellular levels of amyloid beta-protein (A β), and phosphorylation of tau protein.¹ HSV1 DNA is common in amyloid plaques in AD and HSV1 binding proteins are increased by 11-fold to 15-fold in amyloid plaques and neurofibrillary tangles.⁸ In an AD autopsy study, 90% of amyloid plaques contained HSV1 DNA and 72% of HSV1 DNA was plaque associated. In contrast, aged normal brains contained less plaques and only 24% of HSV1 DNA was plaque associated.⁹ HSV2 has effects similar to HSV1 on amyloid and tau protein.¹⁰ HSV1 proteins are present in hippocampal neurons of mice infected intraperitoneally with HSV1, indicating that blood-borne transmission may occur with HSV1 and HSV2 and account for the 10% of cases of herpes simplex encephalitis (HSE) found to be caused by HSV2.¹¹ In a cell-culture model, the antiviral medications acyclovir, penciclovir and foscarnet reduced HSV1 particles and A β and phosphorylated tau (p-tau) accumulation.¹² These findings support an association between HSV and AD and suggest that antiviral medications may be therapeutic.

Reactivation of the latent HSV1 virus in the trigeminal ganglion, which is the primary reservoir for HSV1 during latency, can occur decades after the original infection with retrograde axonal transport of HSV1 particles infiltrating the locus coeruleus and then progressing to the temporal lobe.¹³ Stress, decline in immune function and increased blood-brain barrier breakdown with age may predispose individuals to HSV reactivation.¹ In patients with HSV seropositive, viral DNA was detected from nearly all combined postmortem tissue samples of trigeminal and olfactory ganglia.¹⁴ Dendritic nerve terminals of olfactory receptor neurons are directly exposed, and viruses can enter the brain via olfactory neurons.¹⁵

HSE (causative agent HSV1 in 90% of cases; HSV2 in 10% of cases) occurs in 2–4 per 500 000 people.¹⁶ In HSE, the virus infiltrates limbic structures, particularly the hippocampus and frontal lobes, and the consequent memory loss, cognitive deficits, mood and personality changes are similar to those in AD, including the long-term sequelae of anterograde memory loss, deficits in working memory and visual object recognition, anosmia and dysphasia.¹⁶ The majority of HSE cases occur in people over 50 years old, possibly because blood-brain barrier disruption with ageing supports viral entry. Clinically, antiviral treatment with acyclovir or valacyclovir is effective in reducing mortality and severity of cognitive deficits in HSE.¹⁶

HSV and cognitive impairment

The majority of older adults have had HSV infection during their lifetimes.¹⁷ Reactivation of HSV seropositivity immunoglobulin M (IgM) has been shown to correlate significantly with incident AD,¹⁸ and the anti-HSV1 IgG antibody avidity index has been shown to be higher in amnesic mild cognitive impairment than healthy controls.¹⁹ ORs for the association between HSV1 seropositivity and AD, compared with HSV seronegative individuals, range from 1 to 3 across studies,^{18 20} with an OR of 2 reported in two Swedish cohorts.^{21 22} In a series of 240 adults, HSV1 seropositivity was associated with a lower neuropsychological test battery score and an 18-fold increase in severe impairment in delayed memory.²³ In a Finnish study of 383 home-dwelling patients with cardiovascular disease, seropositivity for HSV1, HSV2 or cytomegalovirus was associated with lower Mini Mental State Exam (MMSE) scores and with decline in MMSE and Clinical Dementia Rating (CDR)²⁴ scores during 1 year of follow-up. In that study, with 0 to 1 viral seropositivities as reference, HRs for 2 and 3 viral seropositivities were 1.8 (95% CI 0.9 to 3.6) and 2.3 (95% CI 1.1 to 5.0), respectively.²⁵ In patients with psychotic disorders, impaired neurocognitive test performance has been associated with HSV1 seropositivity.^{26 27} In summary, the associations between prior exposure to HSV, particularly HSV1, and cognitive impairment are detectable in healthy older adults, patients with cardiovascular disease, and patients with psychotic disorders.

Apolipoprotein E (apoE) ϵ 4 genotype is a well-established risk factor for AD. In animal models, during acute infection with HSV1, apoE ϵ 4 seems to facilitate HSV1 latency in the brain much more than apoE ϵ 3 and is more efficient than apoE ϵ 3 in promoting viral colonisation of the brain.²⁸ In the brains of patients who had AD, apoE ϵ 4 was shown to be more common in HSV1-positive than HSV1-negative individuals, and apoE ϵ 4 was more common in people with recurrent cold sores than people without cold sores.²⁹ These findings suggest that individuals with the apoE ϵ 4 allele may be more susceptible to the effects of HSV in the brain.

Antiviral treatment with valacyclovir

Valacyclovir, a widely used generic antiviral medication, is approved for the treatment of HSV1, HSV2, herpes zoster and chickenpox.³⁰ No other antiviral drug, including acyclovir (intravenous), famciclovir and ganciclovir, is superior to valacyclovir in the treatment of HSV1 and HSV2 infections.

Acting as an oral pro-drug, valacyclovir is converted in vivo to acyclovir, which is then converted by viral thymidine kinase into its acyclovir monophosphate (acyclo-GMP) and acyclovir triphosphate (acyclo-GTP) forms. Acyclo-GTP is a potent inhibitor of viral DNA polymerase with 100 times higher affinity for viral than cellular polymerase.³⁰ Viral enzymes cannot remove acyclo-GTP from the chain, which results in inhibition of further DNA polymerase activity and consequent chain termination.

Its monophosphate form, acyclo-GMP, also incorporates into viral DNA, leading to chain termination. Therefore, valacyclovir leads to the death of infected cells without affecting the DNA of non-infected cells; hence, its side effect profile is benign.

Valacyclovir has been tested in multiple sclerosis (MS) with equivocal results. MS lesion size on brain MRI scan and clinical disability were outcomes; cognition was not assessed in the two MS trials.^{31 32} In a randomised, double-blind, placebo-controlled, 18-week trial in 24 patients with schizophrenia with positive HSV1 titres, valacyclovir 3 g/day was superior to placebo with effect sizes of 0.79, 0.97 and 1.14 for tests of working memory, verbal memory and visual object memory, respectively.³³ These results are promising for the treatment of cognitive deficits in schizophrenia, specifically in individuals with HSV seropositivity. Valacyclovir also improves cognition in patients with HSE, but controlled trials are lacking.¹⁶

Valacyclovir has a generally benign safety profile with minimally higher rates of headache and dizziness compared with placebo in controlled treatment trials.²⁸ In the post-marketing phase, initial double-blind followed by intermittent and continuous valacyclovir treatment for up to 7 years showed no major side effects.^{34 35} Hallucinations, delirium and seizures can occur in less than 1% of patients with renal failure who are taking valacyclovir.³⁶

The recommended oral dose of valacyclovir for peripheral acute HSV infections is 1–3 g daily, and bioavailability is 54%.³⁰ For long-term HSV suppression, the recommended dose is 1 g daily. In the schizophrenia pilot trial that showed superiority for valacyclovir compared with placebo, the efficacious dose was 3 g daily.³³ The difficulty in obtaining efficacy in AD in nearly all trials to date led to the choice of a flexible dose range of 2–4 g daily for Valacyclovir Treatment of Alzheimer's Disease (VALAD). The dose range of 2–4 g daily is safe, increases the chances of finding efficacy, and is well below the dose of 8 g daily reported to be toxic in patients with HIV infection.³⁶ In patients with MS, HSE and healthy controls, there is high brain penetration with a largely linear relationship between oral dose of valacyclovir and cerebrospinal fluid (CSF) acyclovir levels. In MS, sustained CSF acyclovir levels persist for at least 6 months with continuing valacyclovir treatment.³⁵ Based on the literature, CSF acyclovir levels are likely to be in the 3–6 µM range with oral valacyclovir doses of 2–4 g daily.³⁷

This study will evaluate whether antiviral treatment with valacyclovir can improve cognition and function in patients with HSV seropositive with mild AD in a randomised, double-blind, placebo-controlled, 78-week phase II proof-of-concept (POC) trial.

METHODS AND ANALYSIS

Study design features and rationale

VALAD is a phase II, POC, randomised, double-blind, placebo-controlled, 18-month treatment trial of 130 patients (65 valacyclovir and 65 placebo) with mild AD

and antibodies to HSV1 or HSV2. Capsules filled with lactose are used in the placebo condition of the trial. The goal is to evaluate valacyclovir, a repurposed generic antiviral drug, as a treatment for AD and to evaluate its effects on biomarkers of AD in relation to treatment effects.

Role of sponsor

The study is funded by a National Institute on Ageing (NIA) research grant and supervised by an NIA-approved Data Safety Monitoring Board.

Recruitment, eligibility and consent

The Memory Disorders Centre at New York State Psychiatric Institute (NYSPI) and the Behavioural Neurologists' practice group at New York Presbyterian Hospital, both of which are linked to the Columbia University Alzheimer's Disease Research Centre, are the main sources of recruitment. Over 500 new patients are seen annually across these locations. Furthermore, referrals accrue from local senior centres and assisted living facilities, and self-referrals via clinicaltrials.gov and advertising. New York University (NYU) Medical Centre is a second site that recruits eligible patients presenting to their Memory Disorders Programme. NYU conducts all clinical assessments, including neuropsychological testing, for patients at their site, but the MRI, positron emission tomography (PET), lumbar puncture (LP) and relevant blood draw procedures for their patients are conducted at Columbia University Medical Centre (CUMC).

The total sample size of 130 patients requires a recruitment rate of 38 patients annually. HSV seropositivity evaluated at screening is required for inclusion. Based on estimated 60%–65% HSV seropositivity in older adults,¹⁷ each year 60 potentially eligible in-person screenings are needed to achieve the goal of 38 recruited study participants.

Eligibility criteria

Detailed inclusion/exclusion criteria are described in box 1. Adults with a diagnosis of probable AD by NIA clinical criteria are eligible.³⁸ Patients with MCI and CDR=0.5 are also eligible if they have prior evidence of AD neuropathology from either positive amyloid PET scans or an AD CSF profile of low Aβ with high total tau (tau, p-tau) levels based on the Aβ42/t-tau index (ATI, calculated as Aβ42/(240+1.18 t-tau)) with ATI <1.0 and p-tau >61 pg/mL being indicative of AD. We adopted this approach because MCI is heterogeneous, and we wanted to include only those patients with MCI with amyloid and tau biomarkers of AD neuropathology. We are interested in the effects of the antiviral drug valacyclovir on cognition and function as clinical outcomes, and on amyloid and tau as biomarker outcomes, based on the basic science literature on the effects of antiviral drugs like valacyclovir on amyloid and tau and other measures that are affected by HSV.

Box 1 Inclusion/exclusion criteria

Inclusion criteria

1. Adults, male or female. Females must be postmenopausal defined as 12 consecutive months without menstruation.
2. Diagnosis based on NIA diagnostic criteria: probable AD with CDR=1, or CDR=0.5 (questionable dementia) with biomarker evidence of AD either by amyloid PET scan or CSF evidence of AD as defined by a positive A β , tau and phospho-tau protein profile. This criterion applies to patients with these results already available prior to recruitment, and these tests are not required or done as part of screening.
3. Folstein MMSE score of 18–28 (inclusive) out of 30.
4. A family member or other individual who is in contact with the patient and consents to serve as informant during the study.
5. Patient retains capacity to consent for him/herself or retains the capacity to identify a surrogate/caregiver who will consent on his/her behalf.
6. At screening, patients must test positive for serum antibodies to HSV1 or HSV2 (IgG or IgM). If the test is rated 'equivocal', blood will be drawn again within 6 weeks for a repeat test. If the test is equivocal or positive, the patient will be eligible.
7. Use of cholinesterase inhibitors and memantine is permitted. Doses of these medications need to be stable for at least 1 month prior to study entry. Psychotropic medications (other than benzodiazepines prescribed at a dose equivalent of 2 mg lorazepam daily or higher) and other medications prescribed for medical reasons will be permitted throughout the trial.

Exclusion criteria

1. Caregiver is unwilling or unable, in the opinion of the study physician, to comply with study instructions.
2. Patient has dementia predominantly of the non-Alzheimer's type, including vascular dementia, frontotemporal dementia, Lewy body dementia, substance-induced dementia.
3. Modified Hachinski scale score greater than 4.
4. Current clinical diagnosis of schizophrenia, schizoaffective disorder, other psychosis, bipolar disorder or current major depression by Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria. Prior history of major depression will not be exclusionary because 25% of older adults have a lifetime history of major depression.
5. Active suicidal intent or plan based on clinical assessment.
6. Current or recent (past 6 months) alcohol or substance use disorder (DSM-5 criteria).
7. Current diagnosis of other major neurological disorders, including Parkinson's disease, multiple sclerosis, Huntington's disease and amyotrophic lateral sclerosis.
8. Clinical stroke with residual neurological deficits. MRI findings of cerebrovascular disease (small infarcts, lacunes, periventricular disease) in the absence of clinical stroke with residual neurological deficits will not lead to exclusion.
9. Acute, severe, unstable medical illness. For cancer, patients with active illness or metastases in the last 12 months will be excluded, but past history of successfully treated cancer will not lead to exclusion.
10. Sitting blood pressure >160/100 mm Hg.
11. Renal failure as determined by an estimated GFR <44 mL/min/1.73 m².
12. Serum vitamin B12 levels below the normal range.
13. Patients with thrombotic thrombocytopenic purpura/haemolytic uremic syndrome will be excluded (specific exclusion for valacyclovir as per FDA package insert).

Continued

Box 1 Continued

14. Use of benzodiazepines in lorazepam equivalent doses equal to or greater than 2 mg daily.
 15. For patients consenting to LP (estimated 33% of sample), this procedure will be conducted if there is no lower spinal malformation or specific contraindication to LP such as taking prescription anticoagulants.
 16. Metal implants and pacemaker that are not deemed to be compatible by MRI staff, and claustrophobia to the degree that the patient refuses MRI.
 17. Radiation exposure in the prior 12 months that is above the permitted threshold. This will be determined by the study physician and PET Center staff based on questionnaires and medical records.
 18. Severe vision or hearing impairment that would prevent the participant from providing a valid performance on neuropsychological testing.
 19. Olfaction component: current upper respiratory infection (patient tested as soon as this improves), current smoker >1 pack daily (past smoking has been shown not to affect UPSIT scores, UPSIT score <12/40 (10 out of 40 is scored by chance in this multiple-choice test) indicating congenital anosmia. Patients excluded from the olfaction component participate in the main protocol and all other study procedures.
- A β , amyloid beta-protein; AD, Alzheimer's disease; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; FDA, Food and Drug Administration; GFR, glomerular filtration rate; HSV1, herpes simplex virus-1; LP, lumbar puncture; MMSE, Mini Mental Status Examination; NIA, National Institute on Ageing; PET, positron emission tomography; TSH, thyroid stimulating hormone; UPSIT, University of Pennsylvania Smell Identification Test.

Estimated distribution of patients is 55% women with 70% non-Hispanic White, 15% Hispanic, 12% African American, and 3% Asian in origin.

Exclusion criteria include major psychiatric and neurological disorders (box 1). Patients with cerebrovascular lesions identified on MRI but without clinical deficits due to stroke are included. Valacyclovir is excreted through the kidneys. A study of over 76 000 patients taking acyclovir or valacyclovir showed no increase in kidney disease.³⁹ Valacyclovir also has no significant interactions in patients on diuretics,⁴⁰ but to be conservative, we will exclude patients with estimated glomerular filtration rate (GFR) <44 mL/min/1.73 m². We will use the cut-point of 44 rather than the typical cut-point of 60 because the age and gender correction for estimated GFR leads to the majority of women above 85 years having a GFR <60.⁴¹

If a patient is receiving a cholinesterase inhibitor and/or memantine to treat AD, the dose needs to be stable for 1 month prior to study entry. Psychotropic and other medications will be permitted; valacyclovir has no significant drug–drug or drug–food interactions.³⁰ All medication changes during the trial will be documented using the National Alzheimer's Coordinating Centre (NACC) rating form.

Length of clinical trial

The 78-week (18-month) duration per patient provides sufficient duration to evaluate efficacy and safety and is consistent with industry and National Institute of Health supported phase II trials in AD. Total study duration is 5 years.

Randomisation

The NYSPI pharmacy will make up valacyclovir 500 mg tablets and placebo into identical-looking capsules. The randomisation sequences will be balanced in blocks of random size (2, 4) to reduce the likelihood of clinicians guessing the next patient's treatment. Separate randomisation tables are created for patients from Columbia and NYU.

Treatment regimen

Valacyclovir or placebo will be started at 2 g daily (1 g twice daily) and then increased by 1 g daily every 2 weeks until 4 g daily or the maximum tolerated dose is reached, which will be maintained for the rest of the trial. Pills will be 500 mg each to reduce difficulties in swallowing the large 1 g pill. Patients who cannot tolerate valacyclovir 2 g per day (or matching placebo) will discontinue treatment but will be followed at all time-points under the intent-to-treat (ITT) principle. Pill counts will determine adherence. Non-adherence will lead to counselling of patients/caregivers and will not lead to study termination.

Dropout is estimated at 15%. In patients who discontinue study medication, the reason for early exit is documented and study visits continue at scheduled time-points per the ITT principle.

Blindness of raters

Raters remain blind to randomised treatment condition (valacyclovir or placebo). The blind is not broken during the entire trial. In case of clinical emergency, the blinded treatment (valacyclovir or placebo) is continued if possible. Treatment interruption for up to 6 weeks is allowed, typically for intervening medical/surgical conditions.

Study measures

At screening, blood is drawn to assess complete blood count with differential, electrolytes, kidney functions, liver functions and other tests (Sequential Multiple Analysis—Computer), folate and vitamin B12 levels, thyroid functions and serum viral antibodies.

Required visits and procedures are listed in [table 1](#). A telephone assessment can replace the in-person 2-week time-point and 4-week time-point assessments. These assessments are to query for possible side effects of valacyclovir, and during these 4 weeks the dose is increased from the starting dose of 2 g daily to 4 g daily or the maximum tolerated dose.

Alzheimer's Disease Assessment Scale-Cognition (ADAS-Cog11, modified version) includes tests of attention, episodic verbal memory, non-verbal memory and naming.⁴² ADAS-Cog has been used in essentially all

Food and Drug Administration (FDA) registration trials in AD. ADAS-Cog has three different/equivalent word list versions for verbal memory that are given sequentially to reduce practice effects.

Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) is a research measure of daily functioning in AD that has been used in several AD trials, and it will be administered to the caregiver.⁴³ It includes Instrumental Activities of Daily Living that are affected in mild AD.

The Clinician's Interview Based Impression of Change (CIBIC)-plus (range 1–7), a global measure of improvement, is a reliable and well-validated instrument used in AD trials.⁴⁴

CDR will be used to score six domains (boxes); sum of boxes is an exploratory outcome.²⁴ Overall score: 0=none, 0.5=questionable, 1=mild, 2=moderate, 3=severe dementia.

The study physician will complete the NACC (Version 2) Forms that take 35–40 min and include history, neurological exam with assessment of Parkinsonian signs, psychiatric assessment that includes the short version of the Geriatric Depression Scale and the Neuropsychiatric Inventory, Functional Activities Questionnaire (administered to the caregiver), vascular risk factors and the modified Hachinski ischaemic scale.⁴⁵

Neuropsychological test battery (based on NACC-Uniform Data Set Version 3): Montreal Cognitive Assessment, Number Span Test Forward and Backward, Category Fluency for animals and vegetables, Craft Story21 immediate and delayed recall, Benson Complex Figure Immediate and Delayed Recall, Trail Making A and B, Multilingual Naming Test, Verbal Fluency F and L.⁴⁵ This test battery is given for diagnostic purposes at baseline, 52 and 78 weeks.

Safety Monitoring includes vital signs, physical examination, laboratory tests and contact with the patient's primary care physician as needed. Adverse events (AEs) are rated as mild, moderate or severe and on the likelihood of being due to valacyclovir.

Detection of serum antiviral antibodies against HSV1 surface protein gG-1 is 100% specific and 98% sensitive for exposure to HSV1.⁴⁶ Repeated reactivation leads to lifelong elevation of viral titres, but they do not indicate duration or timing of HSV exposure. We obtain serum antiviral antibodies to HSV1 and HSV2 with IgM and IgG from standardised assays performed by Quest Diagnostics.

We conduct LP with the 22-gauge Sprotte needle, as specified on the Alzheimer's Disease Neuroimaging Initiative (ADNI) two website http://www.adni-info.org/Scientists/doc/ADNI2_Protocol_FINAL_20100917.pdf. At 12 and 78 weeks, the reference laboratory at the University of Alabama will examine CSF for acyclovir levels and plasma acyclovir levels. We also obtain CSF Aβ₄₂ and tau/phospho-tau protein levels, and neurofilament light levels (assay in development), at baseline, 12 and 78 weeks. Samples are sent to the reference laboratory at University of Pennsylvania that conducts these assays

Table 1 Study procedures for the Valacyclovir Treatment of Alzheimer's Disease Trial

Procedure	Screening	0 weeks	2 weeks	4 weeks	12 weeks	26 weeks	52 weeks	78 weeks
Clinical assessment/physical exam/ vital signs	X	X	X	X	X	X	X	X
Diagnosis (Consensus diagnosis at weeks 0, 52, 78)	X	X					X	X
ADAS-Cog11		X			X	X	X	X
MMSE	X					X		
CDR	X				X	X	X	X
ADCS-ADL		X			X	X	X	X
CIBIC-plus		X			X	X	X	X
NACC/UDS		X					X	X
Modified Hachinski (from NACC)	X							
Adverse events		X	X	X	X	X	X	X
Serum anti-HSV antibodies with quantitative IgG and IgM	X							X
SMAC blood chemistry	X				X	X		X
Complete blood count with differential	X				X			X
Plasma acyclovir levels					X			X
*CSF acyclovir levels					X			X
*CSF ABeta, tau, p-tau, neurofilament light		X			X			X
*CSF total protein, glucose, cell count with differential		X						
UPSIT olfaction test		X						X
Apolipoprotein E genotype		X						
MRI scan of brain		X						X
18F-Florbetapir PET scan		X						X
18F-MK-6240 PET scan		X						X

*Estimated 40% of patients (n=52) who agree to CSF studies.

ADAS-Cog 11, Alzheimer's Disease Assessment Scale – Cognitive Subscale 11; ADCS-ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living; CDR, Clinical Dementia Rating; CIBIC-plus, Clinician's Interview-Based Impression of Change Plus Caregiver Input; CSF, cerebrospinal fluid; HSV, herpes simplex virus; MMSE, Mini Mental Status Examination; NACC/UDS, National Alzheimer's Coordinating Center/Uniform Data Set; PET, positron emission tomography; SMAC, Sequential Multiple Analysis- Computer; UPSIT, University of Pennsylvania Smell Identification Test.

for ADNI-related studies. Extra aliquots of CSF and serum samples are stored for possible future research analyses. At baseline, clinical CSF assessment for protein, glucose and cell counts is also obtained.

Apolipoprotein E is genotyped (ε2, ε3 and ε4 alleles) by Lgc. genomics (Beverly, MA; <http://www.lgcgroup.com>) using SNPs rs429358 and rs7412.

The University of Pennsylvania Smell Identification Test (UPSIT) is a reliable, well-validated, 40-item scratch-and-sniff multiple choice odour identification test.⁴⁷ The UPSIT comprises of four booklets, each with 10 odours. Total score is 0 to 40; score is 0 (incorrect) or 1 (correct) for each item.

MRI acquisition sequences follow the ADNI protocol for GE scanners (3T): <http://adni.loni.usc.edu/methods/documents/mri-protocols/>. Using each individual's T1-weighted image, global and regional measures are

derived using the FreeSurfer software package (<http://surfer.nmr.mgh.harvard.edu/>). A set of volumetric regions of interest (ROIs) is defined, and the calculated volume within each region is adjusted for variations in the individual's intracranial brain volume measured by BrainWash (automatic multi-atlas skull-stripping software package).⁴⁸ We process the T1-weighted images at the two time-points using the FreeSurfer longitudinal pipeline to detect small changes over time.

To test the exploratory MRI hypothesis, we will examine the AD 'signature' of average cortical thickness of 9 ROIs examined quantitatively by a cortical surface-based reconstruction and analysis.⁴⁹ The 9 ROIs are medial temporal cortex, inferior temporal gyrus, temporal pole, angular gyrus, superior frontal gyrus, superior parietal lobule, supramarginal gyrus, precuneus and inferior frontal

sulcus. Whole brain cortical thickness will be an additional measure in the VALAD Trial.

PET methods for acquisition, quality control, pre-processing and data analysis have been adopted from ADNI. PET scans are conducted on a Biograph mCT scanner (Siemens Healthcare) at the CUMC Kreitchman PET Centre. De-identified data are evaluated for adequacy; quality control checks include visual inspection, assessment for excessive motion between frames, evaluation of header information for scan protocol adherence, determination of image quality metrics (global correlation, mean square error and absolute error) and comparison with PET imaging data forms. The participant's MRI will be utilised for localisation and to conduct ROI analyses of PET data. FreeSurfer will be used to define ROIs from the structural T1 image.

^{18}F -Florbetapir (Amyvid) is purchased from a local vendor. Participant preparation consists of intravenous catheterisation followed by bolus injection of the tracer (injected activity 10 mCi). PET scans are acquired in dynamic, 3D imaging mode beginning 50 min after injection. Four brain images are acquired, each with 5 min scan time over 20 min. The standardised uptake value (SUV), defined as the decay-corrected brain radioactivity concentration normalised for injected dose and body weight, is calculated. SUV is normalised to cerebellar grey matter to derive standardised uptake value ratio (SUVR). SUVR is determined at the voxel and ROI level; the sum of six ROIs known to show increased amyloid radiotracer uptake in mild AD (medial orbital frontal, anterior cingulate, parietal, temporal, posterior cingulate, precuneus)⁵⁰ will be the main outcome.

For ^{18}F -MK-6240 PET imaging (injected activity 5 mCi), PET scans are acquired from 80 to 120 min after injection. Using similar processing methods to ^{18}F -Florbetapir PET, the main outcome is tau SUVR in the medial and inferior temporal lobes with cerebellar cortex as reference region. Tau aggregation is limited to medial temporal cortex in older adults and does not spread into neocortical regions until after AD symptoms become manifest.⁵¹ We will examine superior temporal, occipital, prefrontal cortex, superior and inferior parietal lobule, and precuneus as exploratory ROIs.

Data management

Data entry will be completed by Programme Managers, Clinical Research Coordinators, and Research Assistants on the study protocol. Data entry/cleaning will be done throughout the project. The data collected in this study will be monitored by the Data Coordinating Centre (DCC) at NYSPI. The unit will work closely with the research assistant/coordinator and the Principal Investigator to facilitate independent auditing of primary subject records. The database will provide reports indicating all modifications that have been made in the database together with paper communications (fax, e-mail) confirming and authorising these modifications. Access to the data system is available only to authorised users,

with multiple levels of security including user ID/password authentication via MS Active Directory overseen by experienced IT personnel. Other authorised users with direct access to the data system will be DCC staff. DCC data-related operations and the SIR/Citrix system have been certified by Columbia University's Information Security Office. The dataset will not be published in a data repository.

Hypotheses

Hypothesis 1. On the ADAS-Cog11 (cognitive measure; 0–78 weeks), patients treated with valacyclovir will show smaller decline than patients treated with placebo.

Hypothesis 2. On the ADCS-ADL (function measure; 0–78 weeks), patients treated with valacyclovir will show smaller decline than patients treated with placebo.

Hypothesis 3. On PET scans (^{18}F -Florbetapir, 0–78 weeks), patients treated with valacyclovir will show less amyloid accumulation than patients treated with placebo on the sum of six ROIs (cerebellar reference) known to show increased uptake in AD: medial orbital frontal, anterior cingulate, parietal, temporal, posterior cingulate, precuneus.

Hypothesis 4. On tau PET scans (^{18}F -MK-6240, 0–78 weeks), patients treated with valacyclovir will show less tau accumulation on the sum of medial temporal and inferior temporal cortex (cerebellar reference) than patients treated with placebo.

Additional measures examined will be apoE ϵ 4 genotype as a potential moderator of outcome, changes in serum antibody levels to HSV1 and HSV2, MRI 'signature' in AD of regional cortical thinning and whole brain cortical thinning,^{52 53} and CSF acyclovir levels to examine plasma/CSF acyclovir correlations. CSF studies will be optional and approximately one-third of the sample is expected to agree to LP.

Statistical analysis and sample size

There are two primary outcome measures (multiple outcome measures): change in ADAS-Cog and change in ADCS-ADL. For multiple outcome measures in this phase II trial, statistical significance on any one measure is meaningful and correction for multiple comparisons is not needed, unlike for co-primary outcome measures.⁵⁴ All other outcome measures are secondary or exploratory.

Outcome measures

1. Primary: Change in ADAS-Cog11 scores from baseline to 78 weeks (Hypothesis 1). 2. Primary: Change in ADCS-ADL scores from baseline to 78 weeks (Hypothesis 2). 3. Secondary: Change in ^{18}F -Florbetapir brain uptake in sum of six ROIs from baseline to 78 weeks (Hypothesis 3). 4. Secondary: Change in ^{18}F -MK-6240 brain uptake in sum of two ROIs from baseline to 78 weeks (Hypothesis 4). 5. Exploratory: change in CIBIC-plus (global impression of change), CDR sum of boxes (cognition +function). 6. Exploratory: change in MRI AD 'signature' for cortical thinning and whole brain cortical thinning, and

serum antiviral antibody levels, and evaluation of apoE $\epsilon 4$ genotype as a moderating factor for the two efficacy outcomes. In the subsample (estimated 40%) that gets CSF studies, we will obtain plasma and CSF acyclovir levels and examine plasma/CSF ratios; we will also obtain CSF $A\beta_{42}$, tau, p-tau for subset exploratory analyses with changes in outcome measures. 7. AEs and serious AEs (SEs).

Statistical analysis

Sample size and randomisation: The sample ($n=130$) will be randomised 1:1 to valacyclovir or placebo with the randomisation sequences balanced in blocks of random size (2, 4) to reduce the likelihood of clinicians guessing the next patient's treatment.

ITT/dropouts and missing data: The primary analyses will be in the ITT sample, that is, all randomised subjects according to treatment assignment. If a subject stops treatment during the trial, we will continue to collect all measures for that subject and use the data in the final analyses. Missing data will be dealt with by using generalised linear mixed effects models under the 'missing at random' assumption. For MRI, PET (^{18}F -Florbetapir and ^{18}F -MK-6240), antiviral antibodies, CSF $A\beta$ and tau/phospho tau outcomes with one pre-measure and post-measure, inverse probability weighting of cases with complete data will be used where weights are based on the probability of a subject being a completer versus a dropout. Sensitivity analysis will provide a range of plausible effect estimates that could arise due to non-ignorable missing data.

Hypothesis testing

Hypotheses 1 and 2. We will use generalised linear mixed effects models of cognitive and functional measures collected repeatedly at specific time-points (table 1). Age and gender will be included as control variables if there is any evidence of randomisation imbalance. Cholinesterase inhibitors and/or memantine that are permitted during the trial may be related to cognitive outcomes; we will additionally control for their presence or absence. Hypothesis 3. Patients on valacyclovir will demonstrate less increase (less accumulation) in ^{18}F -Florbetapir uptake (sum of 6 ROIs; SUVR) than patients on placebo. We will use analysis of covariance with the pre-post change in ^{18}F -Florbetapir uptake as an outcome, baseline ^{18}F -Florbetapir uptake as a covariate, treatment condition as a predictor, and baseline age and gender as additional control variables if warranted. Hypothesis 4. Analyses similar to Hypothesis three will be conducted with the data from the sum of two ROIs (SUVR) obtained using MK-6240 PET.

Statistical power

Power analyses for Hypothesis 1 (ADAS-Cog11). The sample size is 130 (65 in each arm); up to five measurements (0, 12, 26, 52 and 78 weeks) are obtained for each participant. We assume that 2.5% dropout between

baseline and 12 weeks, 2.5% of the remaining drop out between 12 and 26 weeks, 5.5% of the remaining drop out between 26 and 52 weeks, and 5.5% of the remaining drop out between 52 and 78 weeks, that is, total 15% dropout by 78 weeks. We conducted power analysis using the Repeated Measures and Sample Size (RMASS) program for longitudinal studies and derived the smallest effect size that can be detected at 78 weeks with 80% power. Assuming the correlation between repeated measures is $r=0.3$ (moderate correlation), and the variance of random intercept and random slope is set to be 1 and 0.2, respectively, the smallest detectable effect size is $d=0.50$ with 80% power. In mild AD, ADAS-Cog11 decline is estimated at 10% annually and at 15% over 18 months.⁵⁵ We hypothesise that the valacyclovir-treated group will show no decline (0%) and the placebo-treated group will show 15% decline over 18 months. Assuming a baseline mean ADAS-Cog11 score of 20 in subjects with mild AD, a 15% decline corresponds to a 3-point difference on ADAS-Cog11 scores between the valacyclovir and placebo groups by week 78. Assuming a SD in ADAS-Cog11 scores of 6.0, this yields a hypothesised effect size of 0.50, which corresponds to 80% power.

This medium effect size is clinically meaningful given the paucity of effective therapies in AD. A small-to-medium effect size (0.30–0.45) for either efficacy measure may not reach significance but still provide enough signal to warrant a phase III trial. For Hypothesis 2, the detectable effect size for ADCS-ADL will be similar to that for Hypothesis 1. For Hypothesis 3 (^{18}F -Florbetapir PET) and for Hypothesis 4 (^{18}F -MK-6240 PET), with $n=55$ completers per group (15% attrition), the minimum detectable effect size of the group difference is $d=0.54$, that is, if changes differ by 0.54 SD between treatment groups, we will be able to detect such difference with 80% power for a two-sided test at the significance level of 0.05. For the exploratory moderator hypothesis, the minimum detectable interaction effect size is Cohen's $f^2=0.07$ (a small-to-medium effect size) with 80% power at a significance level of 0.05, that is, if group X moderator interaction explains more than 6.8% of the variance (Cohen's $f^2 = (R^2/(1-R^2))$) we can detect such effects.

Plans for phase III development. In this phase II study, if there is a significant effect favouring valacyclovir over placebo on one or both of the efficacy outcomes, we will proceed to a larger phase III trial. If the effect size is small to medium (0.30–0.45) for one or more efficacy outcomes, even if non-significant we will still proceed to a phase III trial because of the compelling need for new treatments in AD. If there is a lack of efficacy but a significant effect on either of the two PET biomarker targets, we will proceed to a phase III trial with protocol changes, if needed. Positive results may also lead to future evaluation of valacyclovir's disease-modifying effects in MCI/pre-MCI.

Patient and public involvement

Patients who participate in the research will be referred by physicians or referred from other sources, including self-referral, for their initial screening visit. Patients will not be involved in study design, study recruitment or conduct or dissemination of study results. Patients will not be invited to comment on the study design and were not consulted to develop patient-relevant outcomes or interpret the results. Patients will not be invited to contribute to the writing or editing of this document for readability or accuracy.

Ethics and dissemination

All patients will be presented with the IRB-approved informed consent form (online supplementary file). The caregiver/study partner also signs a consent form that describes their role in the study. Local IRB and state regulations for consent are followed for Columbia/NYSPI and NYU Medical Centre, respectively. FDA investigational new drug (IND) 136 449 exemption letter for valacyclovir, and FDA IND 139291 approval letter for ¹⁸F-MK6240 PET scans, have been received for this protocol.

As described in the consent form, clinically applicable information will be disclosed to patients, including baseline results for MRI safety read, ¹⁸F-Florbetapir PET radiological rating and CSF results. For outcome measures specified in the hypotheses, results obtained at time-points after baseline will not be disclosed. ApoE genotyping results will not be disclosed.

Data safety and monitoring board

Three independent experts with expertise in conducting clinical trials in AD or antiviral treatment trials in other disorders comprise the NIA-appointed data safety and monitoring board (DSMB). The DSMB reviews all SAEs and participates in a teleconference twice a year to determine safety and if the study should continue, and then provides an actionable report to the Principal Investigator and the sponsor (NIA).

The study results will be disseminated through publications and conference presentations, as well as on public websites, including clinicaltrials.gov.

Significance

In an editorial review, 31 senior scientists and clinicians pointed out that the scientific evidence strongly suggests that microbes may be a major cause of dementia, including AD.¹ The review identified HSV1 as the most likely culprit and suggested an antiviral treatment trial to potentially slow or arrest disease progression in AD. VALAD is the first randomised, double-blind, placebo-controlled antiviral drug treatment trial in AD or any type of dementia. If results are positive and confirmed in a larger phase III trial, it will provide a novel treatment approach for this devastating disease.

Author affiliations

¹Department of Psychiatry, Columbia University, New York, New York, USA

²Division of Geriatric Psychiatry, New York State Psychiatric Institute, New York, New York, USA

³Department of Neurology, Columbia University College of Physicians and Surgeons, New York, New York, USA

⁴Department of Neurology, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, New York, USA

⁵Department of Biostatistics, Columbia University Medical Center, New York, New York, USA

⁶Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York, USA

⁷Department of Radiology, Columbia University Medical Center, New York, New York, USA

⁸Center for Cognitive Neurology, Departments of Neurology, New York University Medical Center, New York, New York, USA

⁹Department of Pharmacology, University of Alabama, Tuscaloosa, Alabama, USA

¹⁰Departments of Psychiatry and Bioengineering, University of Pittsburgh Swanson School of Engineering, Pittsburgh, Pennsylvania, USA

Contributors DPD conceived and designed the study. DPD is the principal investigator at the Division of Geriatric Psychiatry, Department of Psychiatry, New York State Psychiatric Institute and Columbia University Medical Center. HAW, WCK, QR, AG, YS, AM, TW, EA, JP, MK, KLB, GHP, DD, KMP, EDH provided editorial comments and contributed to the development and conduct of the study.

Funding The study is supported by grant R01AG055422 from the National Institute on Aging (NIA), NIH, Bethesda, MD, USA. TW is supported by grant AG08051 from NIA.

Competing interests AM, QR, KMP, DAD, WCK, EA, HA, JP, MK have no competing interests. DPD is a consultant to Acadia, Eisai, Genentech, Avanir, Neuronix, Grifols and Bxcel Therapeutics and has grant support from the National Institute of Aging. EDH is a consultant to Biogen and Ionis. TW is a consultant to Alzemed Neuro, Inc. and Grifols and has grant support from the National Institute of Health and the National Institute on Aging. KLB has grant support from Lilly, Amylx and Biohaven. AAG is contracted with Merck. GHP has grant support from the National Institute on Aging. YS is a consultant to Eli Lilly, Axovant, Takeda, and AbbVie, has donated to Piramal Imaging Limited, has grant support from California Walnut Commission, and has a license for the Dependence Scale.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

- 1 Itzhaki RF, Lathe R, Balin BJ, *et al*. Microbes and Alzheimer's disease. *J Alzheimers Dis* 2016;51:979–84.
- 2 Baker HF, Ridley RM, Duchen LW, *et al*. Induction of β (A4)-amyloid in primates by injection of Alzheimer's disease brain homogenate. *Mol Neurobiol* 1994;8:25–39.
- 3 Kane MD, Lipinski WJ, Callahan MJ, *et al*. Evidence for seeding of β -amyloid by intracerebral infusion of Alzheimer brain extracts in β -amyloid precursor protein-transgenic mice. *J Neurosci* 2000;20:3606–11.
- 4 Ball MJ. "Limbic Predilection in Alzheimer Dementia: Is Reactivated Herpesvirus Involved?". *Can. j. neurol. sci.* 1982;9:303–6.
- 5 Ganniclliffe A, Sutton RN, Itzhaki RF. Viruses, brain and immunosuppression. *Psychol Med* 1986;16:247–9.
- 6 Devanand DP. Viral hypothesis and antiviral treatment in Alzheimer's disease. *Curr Neurol Neurosci Rep* 2018;18:55.
- 7 Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, *et al*. Alzheimer's disease-associated β -amyloid is rapidly seeded by herpesviridae to protect against brain infection. *Neuron* 2018;99:56–63.
- 8 Carter CJ. Alzheimer's disease plaques and tangles: Cemeteries of a pyrrhic victory of the immune defence network against herpes simplex infection at the expense of complement and inflammation-mediated neuronal destruction. *Neurochem Int* 2011;58:301–20.

- 9 Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *J Pathol* 2009;217:131–8.
- 10 Kristen H, Santana S, Sastre I, et al. Herpes simplex virus type 2 infection induces AD-like neurodegeneration markers in human neuroblastoma cells. *Neurobiol Aging* 2015;36:2737–47.
- 11 Burgos JS, Ramirez C, Sastre I, et al. Involvement of apolipoprotein E in the hematogenous route of herpes simplex virus type 1 to the central nervous system. *J Virol* 2002;76:12394–8.
- 12 Wozniak MA, Frost AL, Preston CM, et al. Antivirals reduce the formation of key Alzheimer's disease molecules in cell cultures acutely infected with herpes simplex virus type 1. *PLoS One* 2011;6:e25152.
- 13 Steiner I, Kennedy PGE, Pachner AR. The neurotropic herpes viruses: herpes simplex and varicella-zoster. *Lancet Neurol* 2007;6:1015–28.
- 14 Liedtke W, Opalka B, Zimmermann CW, et al. Age distribution of latent herpes simplex virus 1 and varicella-zoster virus genome in human nervous tissue. *J Neurol Sci* 1993;116:6–11.
- 15 Mori I, Nishiyama Y, Yokochi T, et al. Olfactory transmission of neurotropic viruses. *J Neurovirol* 2005;11:129–37.
- 16 Hokkanen L, Launes J. Cognitive outcome in acute sporadic encephalitis. *Neuropsychol Rev* 2000;10:151–67.
- 17 Xu F, Sternberg MR, Kottiri BJ, et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA* 2006;296:964–73.
- 18 Letenneur L, Pérès K, Fleury H, et al. Seropositivity to herpes simplex virus antibodies and risk of Alzheimer's disease: a population-based cohort study. *PLoS One* 2008;3:e3637.
- 19 Kobayashi N, Nagata T, Shinagawa S, et al. Increase in the IgG avidity index due to herpes simplex virus type 1 reactivation and its relationship with cognitive function in amnesic mild cognitive impairment and Alzheimer's disease. *Biochem Biophys Res Commun* 2013;430:907–11.
- 20 Itzhaki RF. Herpes simplex virus type 1 and Alzheimer's disease: increasing evidence for a major role of the virus. *Front Aging Neurosci* 2014;6:202.
- 21 Lövheim H, Gilthorpe J, Johansson A, et al. Herpes simplex infection and the risk of Alzheimer's disease: a nested case-control study. *Alzheimers Dement* 2015;11:587–92.
- 22 Lövheim H, Gilthorpe J, Adolfsson R, et al. Reactivated herpes simplex infection increases the risk of Alzheimer's disease. *Alzheimers Dement* 2015;11:593–9.
- 23 Dickerson F, Stallings C, Sullens A, et al. Association between cognitive functioning, exposure to herpes simplex virus type 1, and the COMT Val158Met genetic polymorphism in adults without a psychiatric disorder. *Brain Behav Immun* 2008;22:1103–7.
- 24 Burke WJ, Miller JP, Rubin EH, et al. Reliability of the Washington University clinical dementia rating. *Arch Neurol* 1988;45:31–2.
- 25 Strandberg TE, Pitkala KH, Linnavuori KH, et al. Impact of viral and bacterial burden on cognitive impairment in elderly persons with cardiovascular diseases. *Stroke* 2003;34:2126–31.
- 26 Dickerson FB, Boronow JJ, Stallings C, et al. Association of serum antibodies to herpes simplex virus 1 with cognitive deficits in individuals with schizophrenia. *Arch Gen Psychiatry* 2003;60:466–72.
- 27 Watson AMM, Prasad KM, Klei L, et al. Persistent infection with neurotropic herpes viruses and cognitive impairment. *Psychol Med* 2013;43:1023–31.
- 28 Burgos JS, Ramirez C, Sastre I, et al. Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA. *J Virol* 2006;80:5383–7.
- 29 Itzhaki RF, Lin W-R, Shang D, et al. Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. *The Lancet* 1997;349:241–4.
- 30 Beutner KR. Valacyclovir: a review of its antiviral activity, pharmacokinetic properties, and clinical efficacy. *Antiviral Res* 1995;28:281–90.
- 31 Bech E, Lycke J, Gadeberg P, et al. A randomized, double-blind, placebo-controlled MRI study of anti-herpes virus therapy in MS. *Neurology* 2002;58:31–6.
- 32 Friedman JE, Zabriskie JB, Plank C, et al. A randomized clinical trial of valacyclovir in multiple sclerosis. *Mult Scler* 2005;11:286–95.
- 33 Prasad KM, Eack SM, Keshavan MS, et al. Antitherpes virus-specific treatment and cognition in schizophrenia: a test-of-concept randomized double-blind placebo-controlled trial. *Schizophr Bull* 2013;39:857–66.
- 34 Tyring SK, Baker D, Snowden W. Valacyclovir for Herpes Simplex Virus Infection: Long-Term Safety and Sustained Efficacy after 20 Years' Experience with Acyclovir. *J Infect Dis* 2002;186:S40–6.
- 35 Vere Hodge RA, Field HJ. Antiviral agents for herpes simplex virus. *Adv Pharmacol* 2013;67:1–38.
- 36 Asahi T, Tsutsui M, Wakasugi M, et al. Valacyclovir neurotoxicity: clinical experience and review of the literature. *Eur J Neurol* 2009;16:457–60.
- 37 Lycke J, Malmeström C, Ståhle L. Acyclovir levels in serum and cerebrospinal fluid after oral administration of valacyclovir. *Antimicrob Agents Chemother* 2003;47:2438–41.
- 38 Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:270–9.
- 39 Lam NN, Weir MA, Yao Z, et al. Risk of acute kidney injury from oral acyclovir: a population-based study. *Am J Kidney Dis* 2013;61:723–9.
- 40 Wang LH, Schultz M, Weller S, et al. Pharmacokinetics and safety of multiple-dose valacyclovir in geriatric volunteers with and without concomitant diuretic therapy. *Antimicrob Agents Chemother* 1996;40:80–5.
- 41 Glassock RJ, Winearls C. Ageing and the glomerular filtration rate: truths and consequences. *Trans Am Clin Climatol Assoc* 2009;120:419.
- 42 Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. *Am J Psychiatry* 1984;141:1356–64.
- 43 Schneider LS. Assessing outcomes in Alzheimer disease. *Alzheimer Dis Assoc Disord* 2001;15:S8–18.
- 44 Rogers SL, Farlow MR, Doody RS, et al. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. *Neurology* 1998;50:136–45.
- 45 Lin M, Gong P, Yang T, et al. Big data analytical approaches to the NACC dataset: aiding preclinical trial enrichment. *Alzheimer Dis Assoc Disord* 2018;32:18.
- 46 Bergström T, Trybala E. Antigenic differences between HSV-1 and HSV-2 glycoproteins and their importance for type-specific serology. *Intervirology* 1996;39:176–84.
- 47 Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania smell identification test: a standardized microencapsulated test of olfactory function. *Physiol Behav* 1984;32:489–502.
- 48 Ardekani BA, Guckemus S, Bachman A, et al. Quantitative comparison of algorithms for inter-subject registration of 3D volumetric brain MRI scans. *J Neurosci Methods* 2005;142:67–76.
- 49 Dickerson BC, Feczko E, Augustinack JC, et al. Differential effects of aging and Alzheimer's disease on medial temporal lobe cortical thickness and surface area. *Neurobiol Aging* 2009;30:432–40.
- 50 Villemagne VL, Doré V, Bourgeat P, et al. Aβ-amyloid and tau imaging in dementia. *Semin Nucl Med* 2017;47:75–88.
- 51 Walji AM, Hostetler ED, Selnick H, et al. Discovery of 6-(Fluoro-¹⁸F)-3-(1H-pyrrolo[2,3-c]pyridin-1-yl)isoquinolin-5-amine ([(¹⁸F)-MK-6240]: a Positron Emission Tomography (PET) Imaging Agent for Quantification of Neurofibrillary Tangles (NFTs). *J Med Chem* 2016;59:4778–89.
- 52 Dickerson BC, Bakkour A, Salat DH, et al. The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. *Cerebral Cortex* 2009;19:497–510.
- 53 Dickerson B, Wolk D. Biomarker-Based prediction of progression in MCI: comparison of AD signature and hippocampal volume with spinal fluid amyloid-β and tau. *Front Aging Neurosci* 2013;5:55.
- 54 Feise RJ. Do multiple outcome measures require p-value adjustment? *BMC Med Res Methodol* 2002;2:8.
- 55 Podhorna J, Krahne T, Shear M, et al. Alzheimer's disease assessment Scale-Cognitive subscale variants in mild cognitive impairment and mild Alzheimer's disease: change over time and the effect of enrichment strategies. *Alzheimers Res Ther* 2016;8:8.