A study of the long term effects of aspirin and other factors on the health of older persons

Protocol

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1. INTRODUCTION

1.1 Summary

ASPREE-XT is a post-treatment, longitudinal observational follow-up study of ASPREE participants [ASPREE Investigator Group, 2013; www.aspree.org; McNeil *et al.* 2017].

1.2 ASPREE Background

ASPREE (ASPirin in Reducing Events in the Elderly) was a joint US/Australian research project that determined whether low-dose aspirin increases healthy life-span, defined as survival free of dementia and disability. ASPREE began in 2010 and completed recruitment in 2014. It was a randomized, double-blind, placebo-controlled, primary prevention trial of daily 100 mg of aspirin in a population of healthy older people in the United States (US) and Australia with a median period of treatment of 4.7 years. ASPREE's primary outcome was length of survival free of dementia and disability and had secondary outcomes encompassing the major health issues related to aging. The trial involving 19,114 persons aged 70 and above (65 years and above for US minorities) was distinctive for its large size, methodological rigor and high participant retention rate in both countries.

ASPREE was unique in the following aspects:

1. It was the first large scale trial to incorporate dementia-free and disability-free survival as a primary outcome. This is now recognized as an appropriate goal of treatment in a primary prevention population of this age group. Within a clinical trial context disability-free survival incorporates an estimate of the overall benefits and risks of aspirin in a single outcome measure.

2. It was one of the first primary prevention trials of aspirin to include cancer incidence, metastases or mortality as a pre-specified endpoint. Recent meta-analyses [Rothwell *et al.* 2010, 2011, 2012] suggests that aspirin has a significant chemopreventive effect becoming evident after a period of 4+ years of aspirin treatment, but questions remain about the magnitude of benefit, and whether it applies to treatment of all cancers and to older people.

3. It did provide information about the impact of aspirin on a range of other conditions (*e.g.* dementia, CVD, stroke, depression, bleeding) where aspirin has been claimed to have benefit (or risks).

The intervention phase of the trial ended in June 2017 after the NIA determined that it was highly unlikely that aspirin would show a benefit on the overall primary outcome within the planned 5-year time frame.

1.3 ASPREE Strengths

For a study involving elderly participants, ASPREE achieved high retention rates and maintained a very high standard of data quality. After a median of 4.7 years of follow up, the percentage of participants who:

- had withdrawn (1.2%) or were out of contact for more than 6 months (2.4%) was very low;
- were still alive (5.4% are deceased) and not withdrawn or lost to follow up, continued to attend annual visits (90%), had phone call annual visits (3%) and had agreed to allow us to continue tracking their medical records without other regular contact (7%).

Apart from the large size of the cohort and very low loss to follow-up, other strengths included a high level of clinical documentation, biospecimens collected at two time points in Australia, and at year three in the United States and that all key outcomes were adjudicated by panels of specialist clinicians.

Enthusiasm from the participants themselves to continue is high, based on their response to a question asked in 2017 about continuing with the study in some form; >95% of respondents in Australia said “yes” to this question.

1.4 Cessation of ASPREE trial medication

ASPREE outcomes were closely followed by a Data and Safety Monitoring Board (DSMB) and the National Institute on Aging (NIA). On reviewing the most recent data, the NIA observed very little difference between the groups taking aspirin or placebo, in length of life free of dementia and disability since the start of the study treatment. The NIA concluded that, after more than 80,000 person years of follow-up had accrued (close to the original number planned for ASPREE), it is extremely unlikely that the study would show a benefit for this, the main study outcome, even if participants continued to take study medication through to the end of December 2017 as originally planned.

Taking this into account, the NIA determined that ASPREE participants be advised to stop taking their study medication, via letters sent in the middle of June 2017.

Although the trial medication was ceased, the study activity was not stopped and ASPREE participants continued with scheduled visits and phone calls through 2017. An observational follow-up phase, that will be known as ASPREE-XT, began in January 2018. This enables the monitoring of possible delayed effects of aspirin treatment, primarily on cancer incidence, metastases and mortality, and the primary and secondary ASPREE- XT outcomes (identical to ASPREE- XT outcomes).

Continuity of contact with study participants is the key to retention of the cohort for any ongoing or future studies.

1.5 ASPREE Results

The primary results of the ASPREE study were published as three manuscripts in the New England Journal of Medicine in September 2018 [McNeil *et al.* 2018a; McNeil *et al.* 2018b; McNeil *et al.* 2018c]. The main outcome was that aspirin initiated in healthy elderly individuals did not prolong disability-free survival over a median 4.7 years but did increase major hemorrhage [McNeil *et al.* 2018a; McNeil *et al.* 2018b]. A higher rate of death from any cause was observed in the aspirin group than in the placebo group, which was attributed primarily to cancer-related death [McNeil *et al.* 2018c].

2. ASPREE-XT (ASPREE-eXTension)

2.1 Aspirin and Cancer Prevention

The ability of ASPREE to answer outstanding questions about aspirin's effect on malignancy provides a strong rationale to follow the ASPREE participants for a further 5+ years. Current evidence suggests that low-dose aspirin may delay the onset of certain malignancies and reduce metastatic spread. However, these chemopreventive effects do not become apparent until after 4-5 years of continuous aspirin therapy [Rothwell *et al.* 2010, 2011, 2012]. Additionally, evidence suggests that the chemotherapeutic effect of aspirin may be dependent on the type, and even subtype, of cancer.

ASPREE has prospectively collected data on cancer incidence throughout the trial using histological and specialist verification to ensure accurate classification, including cancer

subtype, but longer term data collection is needed to quantify the potential magnitude of this effect and to define those cancer subtypes with the greatest benefit. These data have significant public health implications, given the increasing incorporation of aspirin into guidelines for the chemoprevention of colorectal cancer in the general population. With NCI's support, cancer tissue and blood samples for genomic and other analyses have been biobanked; resources that will contribute to understanding of the biological effects of aspirin that might explain this action. The ability to confirm and investigate this delayed effect of aspirin on malignancy requires that the full ASPREE cohort continue to be followed with minimal attrition.

In addition to monitoring the incidence of malignancy within the ASPREE cohort, the opportunity will be taken to observe any other residual effects of aspirin on the endpoints being monitored in the cohort.

2.2 ASPREE Population as the basis for longitudinal aging cohort

As ASPREE participants have been followed within the context of a clinical trial, the study has a high degree of rigor that provides several unique advantages as the basis of a longitudinal cohort study of aging. These include:

1. Absence of dementia at baseline (defined by a 3MS score of >77)
2. Detailed cognitive measures on all participants every 2 years
3. Dementia outcomes adjudicated in all cases
4. Other key health areas including cancer, cardiovascular disease, cerebrovascular disease, frailty, depression and significant bleeding, are adjudicated by expert committees or measured during face-to-face contact
5. Very low loss-to-follow-up (<1%)
6. Extensive medical information & data about personal, environmental & genomic predictors
7. Baseline & 3 year bloods and urines or saliva samples allowing for the assessment of biomarkers

The ASPREE cohort therefore provides a large and unique cohort of elderly individuals where cognitive function has been monitored intensively as well as most other key areas of medical significance. Coupled with the availability of clinical and genomic information, the cohort provides a unique opportunity to study cognition and other major conditions of aging.

2.3 Funding for ASPREE-XT

In 2016, Australia was awarded funds from the National Health and Medical Research Council (NHMRC) through the competitive grants process to fund ongoing contact with ASPREE participants in Australia through 6-month phone calls, to track colorectal cancer events through electronic health records and to collect samples of colorectal cancer tissue. This funding is from 2017-2021, forming the basis for ASPREE-XT.

For the years of 2018-2019, NIH (NIA and NCI) supplied bridge funding to continue study activity, including face-to-face contact and phone calls in the US and Australia. At the invitation of the NIA, the ASPREE investigators submitted a grant application to the NIH seeking funds to continue the follow-up of the ASPREE cohort past the bridge funding in 2018. Funds have since been awarded by the NIH to enable the ASPREE investigators to maintain the cohort and allow the study of multiple aging outcomes for a further five years (2019 - 2024) (see Table 1 below for details).

2.4 Study Aims

ASPREE participants will be followed for a longitudinal, observational follow-up study after the treatment phase to determine:

1. Long term effects of daily aspirin treatment (after an average of 4.7 years in ASPREE) versus placebo on the incidence of cancer, metastases and cancer-associated mortality.
2. Long term effects (after an average of 4.7 years of ASPREE) of daily aspirin treatment versus placebo on the ASPREE primary endpoint and the following ASPREE-XT outcomes: all-cause mortality, dementia, physical disability, mild cognitive impairment, depression and frailty.
3. Long term effects (after an average of 4.7 years of ASPREE) of daily aspirin treatment versus placebo on the incidence of cardiovascular disease including stroke subtypes and major hemorrhage.
4. Demographic, comorbid, genomic and environmental factors that contribute to the maintenance of cognition and other aspects of healthy aging in a highly phenotyped cohort of older adults.

2.5 Study Population

The characteristics of the ASPREE participants at the time they were randomized into ASPREE are described in the ASPREE baseline characteristics paper [McNeil *et al.* 2017].

3. ASPREE-XT STUDY DESIGN

ASPREE-XT is a longitudinal, observational follow-on study of ASPREE participants.

The methodology of ASPREE-XT is based closely on ASPREE [ASPREE investigator group, 2013; www.aspree.org; McNeil *et al.* 2017]. Clinical, neurocognitive and physical function measurements, questionnaires for mood, quality of life, physical ability and collection of personal health, demographic and lifestyle details are the same in ASPREE-XT as those conducted and described for ASPREE.

Since ASPREE-XT is an observational study, the endpoints for ASPREE will be collected and adjudicated in a similar way for ASPREE–XT but will be referred to as ASPREE-XT Outcomes. These will not be divided into primary and secondary endpoints.

In 2018, participants were invited to enroll in ASPREE-XT. At the first ASPREE-XT study visit, or via mail, they were provided with an information sheet and consent form for the study (Table 1). Participants who wish to only be contacted by phone were provided with information and a consent form by mail with health and other study data collected by phone call.

Table 1. Timeline for ASPREE participants willing to continue with ASPREE-XT

Year	2017	Bridging year (2018)	ASPREE-XT (2019 and beyond)
All ASPREE participants are eligible for ASPREE-XT	ASPREE Close-out visits	• Consent to ASPREE-XT • Face to face Annual visit for ASPREE-XT or phone call for data collection • 6 month phone calls for data collection • Medical records collection • Endpoint tracking • Samples of cancer tissue	• Collect outstanding consent • Face to face Annual visit for ASPREE-XT or phone call for data collection • 6 month phone calls for data collection • Medical records collection • Endpoint tracking • Samples of cancer tissue

Annual face-to-face visits and 6 month phone calls will continue beyond 2018, following the securing of additional NIH funds. Phone calls, cancer medical records and cancer tissue collection are funded separately in Australia from the NHMRC until 2021.

3.1 Recruitment

In Australia, ASPREE participants who were attending study visits in 2017 were invited by phone to attend an ASPREE-XT study visit which was at their local GP clinic, community venue or study site. In advance of the appointment, the PICF and some of the ASPREE-XT questionnaires (the LIFE questionnaire, clinical event details, SF-12, cancer screening information, aspirin / smoking / alcohol use, pre-populated participant and nominated contact(s) details for confirmation), were sent by mail to the participant, with a request to bring the completed paperwork along to the study visit. All ASPREE-XT participants will be very familiar with these questionnaires from ASPREE.

ASPREE participants who had chosen to receive study phone calls only in 2017 were sent an invitation to participate in ASPREE-XT, along with a PICF and the questionnaires listed above, with a reply paid envelope. Approximately two weeks later, the participant was contacted to conduct the study visit by phone, including whether the person is willing to participate in ASPREE-XT and a reminder to return the completed PICF if they haven't already done so. In the US, once a site had IRB approval for ASPREE-XT and consent documents, the ASPREE randomized participants were 1) mailed an ASPREE-XT consent form or addendum to sign and return. Once the signed consent was received by site staff, an ASPREE-XT in-person visit or phone call or medical record review was scheduled, based on the participant's desired follow-up status and their ASPREE visit window. Follow-up of consent documents mailed but not returned was monitored by the sites. 2) invited to an in-person ASPREE-XT consenting visit that could also include annual visit activity depending on their ASPREE visit window, or schedule the visit activity for a separate date due to a later ASPREE visit window. In advance of the visit, the consent and some of the ASPREE-XT questionnaires (the LIFE questionnaire, clinical event details, SF-12, cancer screening information, aspirin / smoking / alcohol use), may be sent by mail to the participant with a request to bring the completed paperwork along to the study visit.

Prior to obtaining consent to ASPREE-XT, any participant identified as having dementia will be advised that their nominated next of kin (or other nominated family primary contact) will also be asked to provide written informed consent on behalf of the participant. We respect the rights of

the participant to also sign a consent form themselves.

3.2 Waiver of written consent

In Australia, a waiver of consent applies to the small sub-group of ASPREE participants who have died since the end of the intervention phase of ASPREE (12 June 2017) and who never had the opportunity to consent to ASPREE-XT, and to the participants who previously provided consent during ASPREE to allow access to their medical records, but whom the research staff have been unable to contact since. A waiver of signed consent also applies to those participants who verbally agreed to participate in ASPREE-XT when they were initially contacted by phone, and who subsequently demonstrated their willingness to participate by engaging in scheduled 6 month phone calls, but research staff have been unable to conduct an in-person visit and obtain written consent.

In the US, similar waivers of consent will be sought from the ASPREE-XT sIRB of record.

These waivers of consent will allow the ASPREE investigators to access relevant medical records of the participants in question and obtain supporting documentation that is crucial for outcome adjudication. In the case of deceased participants, the waiver will assist in determining underlying cause of death, and other clinical events of relevance to the study that occurred around the time of the participant's death.

In Australia, a consent waiver also applies to linkages with administrative databases held by the Australian Institute of Health and Welfare (AIHW), other government-based data sets and clinical quality registries. This will provide limited, non-intrusive follow-up of most of the original Australian ASPREE participant cohort – including the small number of participants who withdrew consent during ASPREE, and those who have declined to continue their participation in ASPREE-XT – and will enable basic health outcomes (e.g. cancer diagnoses, admission to residential care) to be ascertained in participants who can no longer be contacted. Participants who have specifically declined to consent to these data linkages will not be included in the waiver.

4. OBJECTIVES

The primary objectives are to determine whether there are long-lasting effects after an average of 4.7 years of treatment with daily low-dose aspirin on the key outcome measures of 1) cancer, metastases and cancer mortality, 2) the composite primary ASPREE-XT outcome of dementia,

disability or death, and 3) secondary ASPREE-XT outcomes of all-cause mortality, dementia, physical disability, mild cognitive impairment, depression and frailty, incidence of cardiovascular disease including fatal and non- fatal stroke, major hemorrhagic stroke, and other major hemorrhage.

An additional objective is to study the impact of demographic, comorbid, environmental and genomic factors on the maintenance of cognition and other aspects of health amongst the elderly. This objective will enable insights that may improve advice available to US and Australian adults to maintain health during their later years.

5. STUDY OUTCOMES

5.1 Main Outcomes

- Death or dementia or persistent physical disability (this composite was the primary endpoint for ASPREE)
- All-cause mortality
- Fatal and non-fatal cancer, excluding non-melanoma skin cancer, and metastases
- Dementia - diagnosed based on DSM-IV criteria [DSM-IV, 1994]
- Physical disability - defined as a confirmed, and persisting for at least 6 months, self-report or appropriate proxy report of ‘a lot of difficulty’, or ‘inability to perform independently’ any one of the 6 Katz basic Activities of Daily Living (ADLs) [Katz & Apkon 1976], or as adjudicated by the Physical Disability Endpoint Adjudication Committee (as described in Appendix 2)
- Mild Cognitive Impairment (MCI; assessed using the Modified Mini-Mental State Examination or 3MS [Teng *et al*. 1987] and other cognitive function measures – see below)
- Fatal and non-fatal cardiovascular events including a) coronary heart disease death, b) non-fatal MI, c) fatal and non-fatal stroke, and d) any hospitalization for heart failure
- Depression; assessed using the CES-D (Center for Epidemiologic Studies – Depression) -10 [Radloff, 1977] questionnaire, or hospitalization for depression
- Major hemorrhagic events
- Frailty [Fried *et al.* 2001]

Note: The terms ‘outcome’ (ASPREE-XT) and ‘endpoint’ (ASPREE) will be used interchangeably in this Protocol.

Each of the endpoints has objective ascertainment criteria (outlined in Appendices 1 and 2) and each will be reviewed by the Endpoint Adjudication Committee (EAC). The cancer, cardiovascular and cerebrovascular endpoints represent the major expected causes of mortality in the study population, whilst dementia, cognitive decline, physical disability and depression impact on the individuals' ability to participate in healthy active life. Hemorrhage is an important endpoint contributing to the evaluation of the risk versus benefits post-aspirin therapy in this population. This endpoint process is based on the ASPREE methodology for endpoint ascertainment.

5.2 Other non-adjudicated outcomes

- Physical function measures: performance-based (including gait speed and hand grip tests) and self-reported (LIFE questionnaire [Pahor *et al.* 2006])
- Additional cognitive function measures: Symbol-Digit Modalities Test (SDMT) [Smith, 1982], Hopkins Verbal Learning Test – Revised (HVLT-R) [Shapirol *et al*. 1999], Controlled Oral Word Association Test (COWAT) [Ross, 2003], and Color Trails [D’Elia, 1996]
- Quality of life (Short Form – 12 or SF-12 [Ware *et al.* 1996])
- Full blood examination results (Australia) / Complete blood count (US); including hemoglobin
- Non-fasting plasma/serum creatinine (for eGFR) and glycosylated hemoglobin (HbA1c)
- Urine albumin: creatinine ratio for the detection of microalbuminuria
- Hospitalization and clinical events of aging

5.3 Outcome Ascertainment

At each annual visit or call, and 6 month telephone contact, the participant will be questioned as to the occurrence of any of the study endpoints over the previous 6 months. Notification of a potential study outcome will trigger the collection of information for event confirmation and adjudication by the EAC. All such events will be recorded on electronic Case Report Forms (eCRF). Confirmation of endpoints will be ascertained by collecting information from the

following sources:

- Details from medical records from the usual treating physician or practice–held medical record
- Medical records from other treating specialist physicians or secondary/tertiary medical care centers
- eCRFs containing standardized questionnaires for the assessment of cognitive function, physical function and quality of life
- In Australia, hospital records/discharge summaries, pathology reports, Medicare (MBS) and Pharmaceutical Benefits Scheme (PBS) records, aged care data sets held by AIHW, National Death Index (NDI), cancer and other registries, Government databases containing information on health and/or vital status. In the United States, hospital records/discharge summaries, pathology reports, public health records, Medicare data, the National Death Index (NDI), and other government databases will be used to obtain health information and/or vital status

6. STUDY COHORT

ASPREE randomized a total 19,114 participants, 16,703 in Australia and 2,411 in the USA. Overall, 56% of randomized participants are female (10,778) and 9% are minorities (1,670). The co-morbidity profile of both Australian and USA participants indicated that the ASPREE population was somewhat healthier than the general population in both countries at baseline, as expected given the eligibility criteria. A summary of the baseline characteristics of the ASPREE participants is reported in the paper [McNeil *et al.* 2017].

6.1 Inclusion criteria

- Randomized participants of the ASPREE study in Australia or the US
- Willing and able to provide informed consent and willing to accept the study requirements, or
- Willing to allow a surrogate or legally authorized representative to provide informed consent

6.2 Exclusion criteria

- Inability or unwillingness to provide informed consent or surrogate / legally authorized representative's consent.

6.3 Participant discontinuation

A participant is free to withdraw from ASPREE-XT at any time. If a participant withdraws from the study altogether (*i.e.* from attending annual visits or follow-up through medical records) or is removed from the study for any reason, this will be recorded in the database.

7. MEASUREMENTS AND DATA MANAGEMENT

7.1 Schedule of study visits

ASPREE-XT measurement and study activity schedule for the bridging period (2018), and 2019 and beyond, is summarized in Table 2.

Table 2. ASPREE-XT Measurement and Study Activity Schedule

	2018		**2019 and beyond**	
Measurement/ Activity	**Annual Visit (XT01)**	**6 month Call**	**Annual Visits (XT02 onwards)**	**6 month call**
Obtain informed consent (or updated consent, if required)	**X** [a]	**X** [ab]	**X** [a]	**X** [ab]
Concomitant medications	**X** [a]		**X** [a]	
Aspirin use	**X** [a]	**X** [b]	**X** [a]	**X** [b]
Demographics, & lifestyle factors* [a]	**X** [a]		**X** [a]	
Family & personal medical history (including cancer) [a]**; cancer screening** [a]	**X** [a]		**X** [a]	
Blood pressure & heart rate, body weight, waist circumference, height [#]	**X**		**X**	
Quality of Life - SF-12 [a]	**X** [a]		**X** [a]	
Assess cognitive function & depression - 3MS; CES-D-10	**X**		**X**	
Additional cognitive function assessments (XT02 onwards) - COWAT, SDMT, HVLT-R, Color Trails	**N/A**		**X**	

Assess physical disability - KATZ ADLs [ab] & LIFE questionnaire [a]	**X** [a]	**X** [b]	**X** [a]	**X** [b]
Assess physical function - Walk test and grip strength	**X**		**X**	
Clinical event reporting - Questionnaire [ab] & medical record review	**X** [a]	**X** [b]	**X** [a]	**X** [b]
Laboratory measures (XT01 only) - Hb, Plasma/Serum Cr & Glucose, uACR	**X**		**N/A**	
Laboratory measures (XT02 onwards) - Plasma/Serum Cr; HbA1c; Full Blood Examination (FBE) (Aus) / Complete Blood Count (CBC) (US), including Hb; uACR	**N/A**		**X**	
Endpoint / Outcome documents collected	**E**		**E**	
Cancer tissue sample collection	**C**		**C**	
Saliva collection	**S**		**S**	

X indicates that all measures are carried out except where superscripts [a] indicates those tests and/or consent forms that may be mailed ahead of the visit to be then brought to the visit or [b] administered by phone; * alcohol and smoking information; **#** height measured at XT03 annual visit and XT06 annual visit only; **E** indicates documents are collected for endpoints at any time there is a trigger; **C** indicates cancer tissue sample collection which occurs at any time during the year after a cancer endpoint is confirmed; **S** indicates collection of saliva from participants who have not yet provided a DNA sample during ASPREE but are willing to do so during ASPREE-XT (Aus participants).

Telephone calls will take place approximately 6 months after the ASPREE-XT visit for purposes of retention and for clinical event reporting. At the 6 month call, participants will be asked specifically about all endpoints, aspirin use and will be administered the Katz ADLs. These calls will take about 15 minutes to conduct.

7.2 Proposed study activity and data collection

Measurements and data will be collected by the research staff after the participant has provided informed consent to participate in the study. The information to be collected at a study visit, by phone call or by US. Mail, includes the following (and is outlined in Table 2):

(a) Basic demographic and lifestyle factors including living situation, smoking history and alcohol use

(b) Medical morbidity – medical record review and questionnaire / participant self-reporting.

(c) Participant reported information about screening for cancer (cancer screening details may be obtained also through medical records provided the information clearly states the test was for cancer screening)

(d) Blood pressure and heart rate: measured in the seated position following 5 minutes of rest using an oscillometric device. Two measurements will be taken 1 minute apart.

(e) Body weight using electronic scale, waist circumference on bare skin with arms down, height (collected at XT03 and XT06 only) in bare feet, with as many body contact points to the wall as possible

(f) Cognitive function and depression: assessed using the 3MS score [Teng *et al.* 1987] and the CES-D-10 [Radloff, 1977] questionnaires. The CES-D is a self-administered questionnaire used to screen for depression. It will be used in association with the 3MS, as depression is a confounder for cognitive function ascertainment, and also to ascertain new onset depression. Additional cognitive assessments will be administered at pre-determined intervals from 2019 and beyond, including the COWAT [Ross, 2003], SDMT [Smith, 1982], HVLT-R [Shapirol *et al.,* 1999], and the Color Trails [D'Elia *et al.,* 1996]

(g) Physical disability: assessed by the participant's self-reported ability, or an appropriate proxy report, to perform the 6 Katz Activities of Daily Living (ADLs), which form a component of the Lifestyle Interventions and Independence for Elders (LIFE) Disability questionnaire [Pahor *et al.* 2006]. The Katz ADLs are best administered face-to-face although they can be administered over the phone.

(h) Laboratory measures: Referral to a local Pathology Provider (in Australia) or direct collection of a blood sample (~12 ml) and urine spot test (minimum of 20 ml). For 2018, the requested measures were creatinine, glucose, hemoglobin, and urine albumin: creatinine ratio (to detect the presence of microalbuminuria). For 2019 and beyond, an amended set of laboratory measures will be requested (non-fasting blood levels of creatinine, hemoglobin within a full blood examination (FBE) (Aus) or complete blood count (CBC) (US), glycosylated hemoglobin (HbA1c), and urine albumin : creatinine ratio).

(i) Short Form (SF) -12 score [Ware *et al.* 1996] – self-administered questionnaire for quality of life.

(j) Physical function testing – timed gait speed test for 3m (8ft) [Guralnik *et al.* 2000] and hand grip strength measured on a grip strength dynamometer [Onder *et al.* 2002].

(k) Concomitant medications, indication and year commenced – participant self-reporting, medical record review and family physician report. Participants will be asked to bring in all of the medications that they currently use or have recently used, including over–the-counter medications, to verify their current medications.

(l) Clinical event reporting. Throughout the year, medical records documents will be collected to support endpoint processing. Triggers from participants' self-report in person or by phone, or a proxy report, along with triggers collected through medical record review, will serve as a basis for requesting relevant documents from various sources.

(m) Cancer tissue retrieval. After an adjudication of cancer endpoint is confirmed, study staff in both countries will seek access to a sample of cancer tissue (see Appendix 6).

(n) Saliva collection (Australia only) for DNA sample will continue in ASPREE-XT for those who still wish to provide a sample (see Appendix 6).

7.3 Participant burden

All participants in ASPREE-XT are familiar with all tests, assessments and measures proposed because the data collection is the same as for ASPREE.

Annual visits conducted in person will require approximately 1 hour and 15 minutes of the participant's time to complete. Annual visits conducted by phone will take around 30 minutes to complete. Questionnaires mailed to the participant (prior to an in-person visit, or following a phone call visit) are estimated to take around 10 to 15 minutes of the participant's time to complete.

7.4 Participant Retention Plan

- Retention will be encouraged by regular phone contact (6 month intervals) between face-to-face visits.

- Contacts details (including at least 2 alternate contacts) will be updated during face-to-face meetings and annual phone call visits. Additional contacts are used in the situation where a participant's contact details change without contacting the study center.

- Newsletters will be sent to family physician co-investigators in AUS and all participants on a regular basis (~2-4 per year) detailing study progress.

If a participant is 'lost to follow-up' despite measures detailed above, the following steps will be undertaken:

- Tracking through third party contacts. Details for up to two additional contacts, who do not reside at the same address as the participant, will be updated during ASPREE-XT.

- Crosschecking with morbidity and mortality registries. In Australia, this may include, but is not limited to, linkage of 'lost to contact' participants with aged care data sets and with the NDI held by AIHW, with other health data sets held by AIHW, and/or other Commonwealth-based or state-based databases and registries containing vital status or health information. In the United States, the National Death Index (NDI) and Medicare data will be used to ascertain mortality status.

- Data linkage through Medicare numbers will provide updated medical information and clinical events in both countries.

- In Australia, the research staff member will be able to also audit practice and hospital records in the event a participant is unable to be contacted.

7.5 Clinical results of significance

An average blood pressure measurement that is higher than 180/105 mmHg, irregular pulse, low 3MS or high CES-D-10 result detected during a study visit will trigger a notification to the participant's GP in Australia and will be provided to the participant's regular treating physician (PCP) in the US if allowed by the participant, who will provide contact details. All pathology results are automatically sent to the participant's nominated GP in Australia who maintains clinical oversight of the participant.

7.6 Data quality control

The ASPREE-XT quality assurance plan is based on that prepared for ASPREE and has been designed to ensure that (a) study staff are fully trained and their performance monitored, (b) there is full compliance with the study protocol and ethical requirements, (c) data collected is complete and accurate, and (d) other procedures (*e.g.* data backup and record storage) are being conducted in accordance with study requirements.

All staff assigned to ASPREE-XT will be provided training covering good research practice, clinical research ethics and procedures. These procedures will be shared in the form of Standard Operating Procedures (SOPs) that will describe, in detail, descriptions of all study procedures. In addition, study CRFs will be required for completion for each visit. All of these materials will be made available via the *AWARD*-XT website. Ongoing training will occur in each country and will be coordinated by each respective Coordinating Center. Trial personnel in supervisory positions will be involved with the training to ensure consistency of procedures between staff in both countries. Ongoing competency will be monitored regarding the various cognitive testing that will be administered by site staff. A 'certification' process will be in place to maintain a high level of testing quality.

The Data Management Center (DMC) will be responsible for data editing that will include checks for missing data and inconsistencies. Site staff will be responsible for completing data edits within a reasonable period of time. The DMC will track quality control measures in a quality control report that will be reviewed by the International Data Management Committee and the International Steering Committee on a regular basis. Such measures will include, but are not limited to, number of participants with missed visits, proportion of study participants retained in the study, documentation for reported study events, proportion of participants with laboratory measures, and deviations from the protocol. If issues with data quality are identified for a particular site or sites, corrective action steps based on remote and/or in-person monitoring will be implemented.

7.7 Data Analysis

The observational ASPREE-XT study will be analyzed by statisticians based at the ASPREE Data Management Center, Department of Epidemiology and Preventive Medicine, Monash University, and the Berman Center for Outcomes and Clinical Research in collaboration with the Chronic Disease Research Group at Hennepin Healthcare, both part of the Hennepin Healthcare Research Institute (HHRI).

Analyses for reporting will be conducted using Stata statistical software release 16 (or a later version if available at the time of undertaking the analyses) or R [R Core Team 2019]. Two-sided p-values will be used and 0.05 will be taken as the cut-off for statistical significance. All confidence intervals (CI) will be reported as 95% CI. There will be no adjustment for multiple comparisons with the protocol pre-specifying the large number of outcomes and subgroups of main interest. Analyses of outcomes not included in the protocol that are undertaken and reported will be labelled in all reports as not being pre-specified.

Specific analyses related to the Aims are detailed in Appendix 3. Table 3 outlines the outcomes to be analyzed under the four aims of ASPREE-XT.

Table 3. Outcomes to be analyzed under the four aims of ASPREE-XT

Outcome	Aim 1 "Long term effects of aspirin on cancer outcomes..."	Aim 2 "Long term effects of aspirin on other outcomes…"	Aim 3 "Long term effects of aspirin on cardiovascular disease and major hemorrhage"	Aim 4 "..Factors that contribute to maintenance of cognition and other aspects of healthy aging."
Disability-free survival (composite of death, dementia & persistent physical disability)		X		X
All-cause mortality		X		
Fatal and non-fatal incident cancer	X			X
Cancer metastases	X			X
Dementia		X		X
Persistent physical disability		X		X
Mild Cognitive Impairment		X		X
Fatal and non-fatal CVD events			X	X
Fatal and non-fatal MI			X	X
Fatal and non-fatal ischemic stroke			X	X
Fatal & non-fatal hemorrhagic stroke			X	X
Major adverse cardiovascular events (MACE)			X	X
Depression		X		X
Major hemorrhage			X	X
Frailty (Fried criteria)		X		X
Frailty deficit accumulation index		X		X

Physical function (gait speed, hand grip, LIFE questionnaire)				X
Cognitive function (Symbol-Digit Modalities Test, Hopkins Verbal Learning Test – Revised, Controlled Oral Word Association Test, and Color Trails)		X		X
Health-related QoL (SF-12)				X
Biomarkers				X
Non-fasting plasma/serum creatinine				X
Glycosylated hemoglobin (HbA1c)				X
Microalbuminuria				X
Hospitalizations				X

Footnote to Table 3. Many of the listed outcomes of interest will have sub-classifications in their own right. For example, fatal and non-fatal hemorrhagic stroke will be sub-classified into a) hemorrhagic stroke as lobar, basal ganglionic, brain stem or b) subarachnoid hemorrhagic stroke; fatal and non-fatal ischemic stroke will be sub-classified as no hemorrhagic transformation, or hemorrhagic transformation with sub-classifications for petechiae or extent of hematoma [Wolfe *et al*. 2018].

7.8 Dissemination and implementation of results

Objectives are:

a) To increase awareness of the ASPREE-XT observational study by disseminating results via repeated messages through scientific and professional channels.

b) To provide physicians, physician's assistants, practice nurses and nurse practitioners with cues to action via distribution of office posters, prescription cards, and other educational materials.

c) To provide study results and any recommendations arising from the study to the professional and scientific community, as well as the public. This will be achieved

through a designated web site that will have the following uploaded: published journal articles, newsletters, presentations, frequently asked questions section, links to other appropriate web sites and downloadable information for personal devices.

8. ADHERENCE TO ETHICAL, REGULATORY AND ADMINISTRATIVE CONSIDERATIONS

8.1 Ethical Considerations

General

This study will be conducted in accordance with the Declaration of Helsinki 1964 as revised in Edinburgh in 2000, and with the National Health & Medical Research Council Guidelines on Human Experimentation.

In Australia, the Alfred Hospital Ethics Committee (ethics #HREC/17/Alfred/198) oversees the ASPREE-XT project as the primary site approver. The project has also been approved by the following secondary ethics committees: the Human Research Ethics Committee (Tasmania) Network (ethics #H0017149); Monash University Human Research Ethics Committee (ethics #12771); and ACT Health Human Research Ethics Committee (Canberra) (ethics #ETH.3.18.037E) and The University of Adelaide Human Research Ethics Committee (ethics #32802), which accept Alfred Hospital Ethics Committee's approval via the National Mutual Acceptance Scheme. Each of these ethics committees previously approved the ASPREE study. These committees will be responsible for ethical oversight of the ASPREE-XT at each of the main hubs.

In the US, based on new National Institute of Health Requirements for multi-site clinical trials, the ASPREE-XT study will use a Single IRB of record.

Information for Participants

Before obtaining consent, the participant, or appointed surrogate, must be informed of the objectives, any benefits or risks, and requirements of the study. Enrolment in ASPREE-XT is only available to ASPREE participants. This means that all ASPREE-XT participants will be already familiar with the planned study measurements and data collection.

8.2 Regulatory Considerations

Financing

ASPREE-XT has received funding from the National Institute on Aging and the National Cancer

Institute (within the US National Institutes of Health) and the National Health and Medical Research Council (Australia).

Disclosure of Conflict of Interest

Full disclosure by all of the key members of the study of their, and their immediate family's, financial relationships with all pharmaceutical and biomedical companies judged to have an active or potential interest in the conduct and outcome of the study will be made annually. Details and conditions of this disclosure are included in Appendix 4.

8.3 Administrative Organization

Individual and Committee responsibilities

The ASPREE-XT International Steering Committee will be responsible for the overall management and conduct of ASPREE-XT including finalizing the protocol, and approving the operational plan for the study. The committee will be chaired by either the US or AUS Principal Investigator or nominee, on an annual rotation as Chair. The committee will consist of the Co-Chairs, members of the International Operations Committee (see below), Principal Investigators from sites in the US and recruiting hubs in Australia, plus individuals with special content expertise who may be invited to join. The International Steering Committee will have conference calls as needed, but at least every 6 months.

International Executive Committee (IEC) is a smaller committee derived from the International Steering Committee. It will consist of the Co-Chairs of the International Steering Committee, two representatives from the US Operations Committee, two representatives from the Australian Operations Committee and the Chair or Co-Chair of the International Data Management Committee. The IEC will meet by teleconference, as needed. This smaller committee must be prepared to meet at short notice if any urgent issues related to the study should arise.

National Operations Committees will be maintained in both countries. Each committee will be responsible for implementing the study, monitoring the progress and ensuring adherence and standardization throughout the study in that country. Each committee will also work to ensure co-ordination, consistency, transfer of data to the Data Management Center and quality control. They will ensure quality control review of laboratory data, clinical measurements and data collection, completeness and entry times of data, site monitoring and source documentation. Each committee will communicate regularly with each other (4-6 teleconferences per year) to ensure consistency of protocol implementation across countries. These Committees may

consist of site Principal Investigators or Project Managers and key research personnel and will report directly to the International Steering Committee.

International Data Management Committee: This committee will meet monthly in order to monitor the establishment and implementation of the ASPREE Data Management System, the reporting activities and to review all quality assurance reports for the study. The IDMC Chair or Co-Chair will report to the International Steering Committee of ASPREE-XT on Data Management Issues associated with the study.

Observational Study Monitoring Board (OSMB): This committee will be appointed by the NIA and will adhere to the OSMB Charter. The responsibilities and operational details of the OSMB are described in Appendix 5.

The OSMB will be provided with data every 6 months or as requested. The OSMB will meet every 6 months, by teleconference or in-person, to review study progress, data quality control, address policy issues and review total mortality data, adverse events, all safety data and monitor the study progress and data quality. The OSMB will provide a follow-up report and recommendations to the NIA. The OSMB has expertise related to the conduct of clinical trials *per se* and in the primary care sector, epidemiology, biostatistics, clinical pharmacology, geriatricians, and cardiovascular disease. Confidentiality will be maintained during all phases of the trial including monitoring, preparation of study results, review, and response to monitoring recommendations.

Endpoint Adjudication Committee: This committee will evaluate individual outcomes blinded to the former randomized ASPREE treatment. Each endpoint will be validated through examination of relevant clinical information. Specialist sub-committees will be appointed, such as the Dementia Adjudication Panel, to adjudicate on specific endpoints with a nominated person as member of the EAC.

Publications, Presentations and Ancillary Studies Committee: This committee will be responsible for implementation of the publication policy (see below), coordination of topics and requests for publication, approval for abstracts and submission to conferences, and receipt of proposals for ancillary studies (see below).

Publication policy

This policy covers all publications and abstracts originating from ASPREE-XT and any sub-study.

Authorship - Manuscripts and abstracts relating to the ASPREE-XT study must include all current members of the International Steering Committee (ISC) using the following formula:

- All publications will be on behalf of 'the ASPREE-XT Study Group'.
- A writing committee will be established for each publication from which a lead author will be identified and responsible for the initial draft of the manuscript
- The lead author will be the first author of the publication
- Subsequent author(s) from the writing committee will be listed according to the amount of input to the writing of the paper.
- All other contributors in last name alphabetical order.
- Members of the ASPREE Steering Committee will be named in description of the ASPREE-XT Study Group in each manuscript
- All clinical site investigators and committee members will be listed on the ASPREE-XT web-site and acknowledged in every publication.

Non-ISC authors utilize the same formula. Disputes about authorship must be notified to the Principal Investigator(s) to be resolved at the next ISC meeting.

Drafts - Initial and major upgraded manuscripts and abstracts must be circulated to all members of the ISC and any other Committee within ASPREE-XT when appropriate. Members will have a time limit, typically a maximum of one week, to send responses.

Ancillary studies

Ancillary studies must be submitted to and approved by the ISC. They are subject to independent sources of funding being procured and must not impact adversely on the main goals and conduct of the trial. Applications should be made to the ISC and submitted by a Principal Investigator with a maximum of a five page summary of the rationale and method and must include a budget and evidence of funding or a strategy for securing said. The ISC must ensure that any ancillary study will not compromise the main study.

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APPENDIX 1. ASPREE-XT MAIN OUTCOMES AND OTHER MEASURES

It is important to note that participants in ASPREE-XT are familiar with all tests, assessments and measures proposed. Data collection for ASPREE-XT is the same as for ASPREE except 3 cognitive assessments were omitted for the first year of visit activity (2018) due to time constraints. These cognitive assessments will be reintroduced in subsequent years along with an additional cognitive assessment (the Color Trails).

Endpoint ascertainment in ASPREE-XT, triggers or reports of events, source documentation to support the case, presentation of cases to the EACs and the criteria / decision rules used by the EACs, will all be the same as for ASPREE. Details of the Endpoint ascertainment procedures for each of the following outcomes are provided in Appendix 2.

1. Main Outcomes

1.1 Composite ASPREE primary outcome

Death from any cause or incident dementia or permanent physical disability.

These categories of disability are defined, respectively, as a) all-cause mortality, b) the assessment of dementia by DSM-IV criteria or c) the onset of 'a lot of difficulty' or 'inability' to perform independently, any one of 6 Katz ADLs.

1.2 All-cause mortality

Death certification and post mortem report. In the absence of a death certificate or post mortem report, hospital or GP records describing the death will be used. National Death Index searches will be conducted in both countries. In Australia, an on-line database (Ryerson Index) will be regularly accessed for information regarding deaths and funeral notices and matched to the ASPREE-XT participants.

1.3 Incidence of fatal and non-fatal cancer (excluding non-melanoma skin cancer) including metastases

1.4 Incidence of all-cause dementia

1.5 Loss of physical ability for activities of daily living (ADL)

1.6 Fatal and non-fatal cardiovascular events

1.7 Mild cognitive impairment

1.8 Depression

1.9 Major hemorrhagic events - *Clinically significant bleeding*

2. Other non-adjudicated measures

2.1 Physical Performance

a) Performance based measures

b) Self- (or proxy-) reported measures (using ADL and IADL Questionnaires)

c) Frailty measures

i. Fried Frailty Index

ii. Deficit Accumulation Score

2.2 Quality of life

2.3 Glycosylated hemoglobin (HbA1c), and non-glycosylated hemoglobin (Hb) within a full blood examination (FBE) (Aus) or complete blood count (CBC) (US)

2.4 Plasma creatinine (eGFR) and Urine Albumin: Creatinine Ratio

2.5 Hospitalizations (for reasons other than Endpoints)

2.6 Derived outcomes

a) Diabetes

b) Hypertension

c) Chronic kidney disease

APPENDIX 2. ASPREE-XT MAIN OUTCOME DEFINITIONS & ENDPOINT ADJUDICATION CRITERIA

(alphabetical order)

CANCER – FATAL AND NON-FATAL

Definitions and processes for adjudication

Fatal and non-fatal cancer is defined as incident non-metastatic cancer (cancer type not present prior to randomization into ASPREE), or incident metastatic cancer. Incident metastatic cancer includes: incident cancer that was metastatic at presentation, incident metastasis of a non-metastatic cancer present at baseline, or incident blood cancer.

Non-melanoma skin cancer is excluded from the fatal and non-fatal cancer end point / outcome, as is local recurrence of a previous cancer.

Specific decision rules developed by the Cancer Endpoint Adjudication Committee (EAC) include:

- Cancers must be histopathologically confirmed, unless histology was not performed, in which case confirmation on imaging or through strong clinical evidence will be accepted (e.g. currently receiving treatment for cancer).
- Where there is no evidence of metastatic disease, participants will be coded as not having metastatic disease.
- Presentation of local (local or regional) nodal disease is not considered metastatic disease, whilst distant nodal disease represents metastatic disease.
- Presentation of metastasis without a prior diagnosis of the primary cancer is considered metastatic on presentation and thus only one cancer end point - 'Metastatic cancer end point'
- Presentation of metastasis within 3 months of the primary diagnosis is considered metastatic on presentation and thus only one cancer end point - 'Metastatic cancer end point'.
- Presentation with metastasis greater than 3 months after the primary diagnosis is considered metastatic spread following an initial non-metastatic presentation. Consequently, the event is linked with two cancer end points: one non-metastatic (i.e. the initial presentation) and one metastatic. Cases of this nature will be presented to the EAC in two parts, once for the initial presentation and once for the metastatic spread. The summary document alerts adjudicators as to the part being presented for adjudication.
- Exception rule: non-melanoma cancers of the skin will be considered as a primary cancer if they are reported as either the primary or secondary cause of death.
- Exception rule: non-melanoma cancers of the skin that progress to metastasis will be considered as a metastatic cancer event.

Source information from clinical case notes and hospital medical records related to these events will be collected, sent to the ASPREE-XT Clinical Event Team and presented to adjudicators on the Cancer EAC. Adjudicators are blinded to participant identity and original

treatment arm for ASPREE. Each blinded case will be sent to two adjudicators and if there is discordance in the outcome, the case will be sent to a third adjudicator for a decision. Any case can be taken to a meeting of the EAC for discussion if an adjudicator needs to seek clarification in interpreting the notes or applying the decision rules.

CARDIOVASCULAR / CEREBROVASCULAR DISEASE

Definitions and process for adjudication of fatal and non-fatal cardiovascular events

Cardiovascular events include a) Coronary heart disease death, b) non-fatal myocardial infarction (MI), c) fatal and non-fatal stroke, d) non-coronary cardiac or vascular death and e) hospitalization for heart failure.

Source information from hospitals/medical centers, treating physicians, death certificates, medical records, hospital information obtained from the next of kin or other family members where relevant will be collected, sent to the ASPREE-XT Clinical Event Team and presented to adjudicators of the Death, Cardiac or Stroke EACs as appropriate. Adjudicators are blinded to participant identity and original treatment arm for ASPREE.

a) Coronary heart disease death is defined as death from MI, sudden cardiac death, rapid cardiac death (death after possible MI), cardiac failure death (with coronary cause) and other coronary death.

- MI - Autopsy or death certificate diagnosis, with definitive or suspected diagnosis of MI within 4 weeks of death.
- Sudden cardiac death - Death occurring within one hour of the onset of new cardiac symptoms (ischemic chest symptoms or sudden collapse) or unwitnessed death after last being seen without new cardiac symptoms, and in each case, without any coronary disease (clinically or at autopsy) that could have been rapidly fatal.
- Rapid cardiac death (death after possible MI) - Death within 1-24 hours of the onset of severe cardiac symptoms unrelated to other known causes. Death in hospital with possible MI (i.e. participants who have had typical ischemic pain and whose ECG and enzyme results fulfil the criteria for definitive MI and in whom there was no good evidence for another diagnosis for the event).
- Cardiac failure death - Death due to heart failure (prior New York Heart Association (NYHA) Class III-IV dyspnea), without any defined non-coronary cause.
- Other coronary death - Any death where the underlying cause is certified as coronary (and where there is no evidence of non-coronary cause of death, clinically or at autopsy).

The Death EAC is responsible for determining if events meet this definition. Time-to-event for coronary heart disease death will be taken as the date of death recorded on death certification.

b) Non-fatal MI is defined according to the American College of Cardiology & European Society of Cardiology definition [Alpert *et al*. 2000] and classified as either acute evolving or recent MI, or established MI.

Criteria for acute, evolving or recent MI include either one of the following:

1. Typical rise in troponin or CK-MB as biochemical markers of myocardial necrosis with at least one of the following:
    - ischemic symptoms;
    - development of pathologic Q waves on the ECG;
    - ECG changes indicative of ischemia (ST segment elevation or depression);
    - coronary artery intervention (e.g. coronary angioplasty).

2. Pathologic findings of an acute MI. Criteria for established MI include either one of the following:

- Development of new pathologic Q waves on serial ECGs. The participant may or may not remember previous symptoms. Biochemical markers of myocardial necrosis may have normalized, depending on the length of time that has passed since the infarct developed.
- Pathologic findings of a healed or healing MI.

The Cardiac EAC is responsible for determining if events meet this definition. Time-to-event for non-fatal MI will be taken as the date of troponin rise for acute, evolving or recent MI, and the date of ECG or pathology report for established MI.

c) Fatal and non-fatal stroke is defined according to the WHO definition as rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery or death) with no apparent cause other than of vascular origin [WHO Task Force, 1989]. This definition excludes cases of primary cerebral tumor, cerebral metastasis, subdural hematoma, post seizure palsy, brain trauma, and transient ischemic attack.

Fatal stroke is defined as any death due to the rapid onset of a new neurological deficit attributed to obstruction or rupture in the intracranial or extracranial cerebral arterial system.

The Stroke EAC is responsible for determining if events meet this definition. Time-to-event for stroke will be taken as the date of first evidence of disturbance of cerebral function.

Confirmed strokes will be further classified as:

- Ischemic stroke (included in cardiovascular end point)
- Ischemic stroke with hemorrhagic transformation (included in cardiovascular end point)
- Stroke type uncertain (included in cardiovascular end point)
- Hemorrhagic stroke (included in major hemorrhage end point)
- Sub-arachnoid hemorrhage stroke (included in major hemorrhage end point)

Ischemic stroke sub-classification - Cerebral infarction can be confirmed by autopsy. The TOAST classification for sub-type of acute ischemic stroke will be utilized, in which both clinical features and ancillary tests (laboratory, radiology, and ultrasonography) are used to categorize five subtypes [Adams *et al*. 1993]

1. large artery atherosclerosis (embolus/thrombosis);
2. cardio embolism (high risk/medium risk);
3. small-vessel occlusion (lacunae);
4. stroke of other determined etiology;
5. stroke of undetermined etiology:
   (a) two or more causes identified;
   (b) negative evaluation;
   (c) incomplete evaluation.

Distinction between ischemic and hemorrhagic stroke can be made only with appropriate imaging as outlined in the table below and described in the ASPREE Statistical Analysis Plan [Wolfe *et al*. 2018]:

	CT	MRI
Ischemic stroke	An area of low attenuation or a normal appearance in the vascular territory that corresponds to the recent	A critically relevant area of increased signal on diffusion weighted imaging, a slight hypointensity with or without mass effect on T1-weighted images, a

	symptoms and signs	bright area of hyper-intensity with or without mass effect on T2-weighted images, or evidence of recent infarction on diffusion weighted MRI
Hemorrhagic stroke	An area of hyperdensity within the brain parenchyma with or without extension into the ventricles or subarachnoid space or, for scans performed beyond 1 week, an area of attenuation with ring enhancement after injection of contrast	An area of hypointensity or isointensity on T1-weighted images or an area of marked hypointensity on gradient echo and T2-weighted images, or by autopsy demonstrating the origin of the hemorrhage as the cerebral parenchyma

Note: Rarer causes and sites of intracerebral hemorrhage such as underlying arteriovenous malformation and spinal cord hemorrhage will be documented.

Hemorrhagic stroke sub-classification – Sub-classification of hemorrhagic strokes will be based on imaging information, as described in the table above. To complement the use of the TOAST classification for thrombo-embolic stroke, the extent of intracerebral hemorrhage will be quantified by assessing hemorrhage site and volume by CT or MRI. Volume is assessed by utilizing the ABC/2 formula with hemorrhage sites as lobar, basal ganglionic or brain stem [Kothari *et al*. 1996].

Sub-arachnoid hemorrhage (SAH) – These will be reviewed by the Stroke EAC. SAH must have satisfied all the criteria above to be considered as stroke. SAH that do not meet the above criteria will be adjudicated as 'Not stroke end point – intracranial bleed present but event did not meet the stroke criteria.' Events with this outcome will be sent to the neurologist on the Clinically Significant Bleeding (CSB) EAC who will determine whether the event meets the CSB criteria.

d) Non-coronary cardiac or vascular death – Health or coronial records of death or sudden death attributable to cardiac-related or vascular-related origins that were not due to coronary or myocardial ischemia will be provided to the Death EAC for consideration. If considered appropriate, other EACs such as the Cardiac or Stroke EACs will adjudicate the event. Such deaths may include those attributed to abdominal aortic aneurysm (AAA) rupture, large vessel atherosclerosis, cardiomyopathy, cardiomegaly, myocarditis, or peripheral vascular disease.

The Death EAC is responsible for determining if events meet this definition. Time-to-event for non-coronary or vascular death will be taken as the date of death recorded on death certification.

e) Hospitalization for heart failure - Hospital discharge diagnosis of heart failure triggers an assessment by the Cardiac EAC. Hospitalization for heart failure is defined as an unplanned overnight stay, or longer, in a hospital environment (emergency room, observation unit or inpatient care) or similar facility. Heart failure is defined as a participant having typical symptoms (e.g. dyspnea, fatigue) that occur at rest or on effort that is characterized by objective evidence of an underlying structural abnormality or cardiac dysfunction that impairs the ability of the ventricle to fill with or eject blood (particularly during exercise). The diagnosis of heart failure may be further strengthened by a beneficial clinical response to treatment(s) directed towards amelioration of symptoms associated with this condition. Where possible, heart failure diagnosis will be confirmed by demonstrated pulmonary congestion or edema on chest imaging. If chest imaging is not available, documented evidence of clinical signs of pulmonary edema (e.g. rales > 1/3 up the lung fields thought to be of cardiac causes), pulmonary capillary

wedge pressure >18 mmHg or B-type natriuretic peptide of >500 pg/ml will be utilized to confirm the diagnosis of heart failure.

The Cardiac EAC is responsible for determining if events meet this definition. Time-to-event for hospitalization for heart failure will be taken as the date of hospitalization. Each blinded case will be sent to two adjudicators and if there is discordance in the outcome, the case will be sent to a third adjudicator for a decision. Any case can be taken to a meeting of the EAC for discussion if an adjudicator needs to seek clarification in interpreting the notes or applying the decision rules.

DEATH (ALL-CAUSE and CAUSE-SPECIFIC MORTALITY)

Definitions and processes for death confirmation and cause of death

Confirmation of death - Reported deaths are considered to be confirmed upon verification with two independent sources (e.g. family or PCP report, or clinical record, or public death notice).

Cause of death - Cause of death is defined as the disease responsible for trajectory to death.

Source information from hospitals/medical centers, treating physicians, government bodies (e.g. Government registers) autopsy reports, death certificates, medical records, and information obtained from the next of kin or other family members where relevant will be collected, sent to the ASPREE-XT Clinical Event Team and presented to adjudicators of the Death EAC.

Adjudicators are blinded to participant identity and original treatment arm for ASPREE. Adjudicators will consider the progression of disease and disability over the course of the study and then assign cause of death based on trajectory to death, as described below:

Cancer Related Death

Death from all incident cancers (excluding non-melanoma skin cancers) confirmed histologically.

Cardiovascular Death

Death resulting from arrhythmia, pulmonary embolism, peripheral arterial disease, or complications of a cardiovascular procedure.

Clinically Significant Bleeding Death

Death from clinically significant bleeding defined as any hemorrhagic event (hemorrhagic stroke, symptomatic intracranial bleeding, or major gastrointestinal bleeding or other extracranial bleeding) that proved fatal.

Coronary Heart Disease Death sub-types

Includes Cardiac Failure, Myocardial Infarction, Rapid Cardiac Death, Sudden Cardiac Death and Other coronary death.

Stroke Death

Death due to the rapid onset of new neurological deficit attributed to the obstruction or rupture in the intracranial or extracranial cerebral arterial system. Stroke will be defined according to the World Health Organization (WHO) definition. This definition excludes primary cerebral tumor, cerebral metastases, subdural hematoma, post seizure palsy, brain trauma and transient ischemic attack.

Other Death

Death due to causes other than those listed above which may include chronic lung disease, dementia, infection, sepsis.

Adjudication of fatal and non-fatal end points will occur independently by disease-specific EACs and results of these adjudications will be made available to those adjudicating cause of death. When relevant records cannot be obtained, cases will be assigned a cause of death based on National Death Index (NDI) International statistical Classification of Diseases and related health problems (ICD)-10 codes.

Time-to-event for death will be taken as the date of death recorded on death certification. Each blinded case will be sent to two adjudicators and if there was discordance in the outcome, the case will be discussed at a meeting and the outcome agreed to by all adjudicators.

DEMENTIA

Definition and process for adjudication

Dementia is defined according to the Diagnostic and Statistical Manual for Mental Disorders, American Psychiatric Association (DSM-IV) criteria [DSM-IV, 1994].

Diagnostic features included:

A. memory impairment and;

B. at least one of the following: Aphasia, apraxia, agnosia, disturbances in executive functioning.

In addition, the cognitive impairments must have been severe enough to cause impairment in social and occupational functioning, the decline must have represented a decline from a previously higher level of functioning, and the diagnosis of dementia should not have been made if the cognitive deficits occurred exclusively during the course of a delirium.

A 3MS (Modified Mini-Mental State examination; scored out of 100) [Teng & Chui, 1987] score of below 78, a fall in 3MS score of more than 10.15 points compared with the individual's base line score after adjustment for age and level of education, a diagnosis of dementia, a report of thinking/memory concerns to a specialist, or prescription of a cholinesterase inhibitor (Australia only, where Pharmaceutical Benefits Scheme supports prescription only after a clinical diagnosis of cognitive impairment) triggers completion of additional and uniformly applied cognitive and functional testing. These tests are conducted by trained and accredited research staff. Collection of ancillary data (laboratory tests and a CT or MRI) will be sought for end point ascertainment.

Source information from dementia assessments, clinical case notes and hospital medical records related to these events will be collected, sent to the ASPREE-XT Clinical Event Team and presented to adjudicators of the Dementia End point Adjudication Committee (EAC). Adjudicators are blinded to participant identity and original treatment arm for ASPREE. Cases will be sent to two adjudicators and if there is discordance in the decision, the case will be taken to a meeting of the EAC that has to be unanimous in the final decision. Any case can be taken to a meeting of the EAC for discussion if an adjudicator needs to seek clarification in interpreting the notes or applying the decision rules.

Time-to-event for dementia will be taken as the time between randomization and the date of the trigger (low 3MS, dementia diagnosis or cholinesterase inhibitor prescription) that is subsequently confirmed as a dementia endpoint by the Dementia EAC.

Sub-classification of Alzheimer Dementia (AD) cases based on the National Institute of Aging – Alzheimer's Association (NIA-AA) Criteria.

Guidelines for review of Dementia due to Alzheimer's disease:

1. Review the case with only the information provided in the Endpoint Notification (EN) document at the time of endpoint adjudication, accepting the original adjudication decision, and assign a sub-classification of either: (1) Probably due to AD, (2) Possibly due to AD, (3) Vascular dementia – not AD, or (4) Not AD.
2. Cases with inadequate information to assign the "Probably due to AD" category are more likely to fit "Possibly due to AD" category because the timeline of disease onset is difficult to define.
3. Cases with a mixed disease presentation can be difficult to categorize, but are more likely to fit the "Possibly due to AD" category. These cases often meet the core clinical criteria

for AD, but evidence of cerebrovascular disease or extensive infarcts or severe white matter hyperintensity burden may also be present [McKhann *et al*. 2011].

Guidelines for review of MCI consistent with Alzheimer's disease:

Utilize the Albert *et al*. (2011) criteria with some additions for review of cases that do not meet the dementia endpoint but may represent MCI.

DEPRESSION

Definition and process for assessment/adjudication

Depression is assessed using the CES-D (Center for Epidemiologic Studies – Depression) 10 questionnaire [Radloff, 1977], or hospitalization for depression (identified through participant self-report, or through medical record review).

A modified 10 questions version of the CES-D is used. The modification omits questions related to lifestyle that are covered by the LIFE questionnaire. A score of 8 or more on the 30-point CES-D 10 questions version is deemed clinically significant [Steffens *et al*. 2002], and a depression endpoint will have been reached automatically. Any such score will be communicated back to the family physician, and would typically result in a clinical assessment.

Cases of elevated (8 or more) CES-D 10 scores will be reported with respect to:

- New onset (incident) depression – defined as an elevated CES-D 10 score at an annual visit where there has been no history of elevated CES-D 10 scores previously since randomization to ASPREE,
- Persistence – defined as an elevated CES-D 10 score at two consecutive annual visits, and
- Recurrence – defined as a CES-D 10 score that was elevated at a previous time point, was not elevated at the subsequent visit, but which has then returned to a score of 8 or more at the most recent annual visit

Any participant discharged from hospital with a principal diagnosis of depression will also be deemed to have reached an endpoint for depression, if adjudicated as such by the Depression EAC. Relevant hospital medical records will be collected, sent to the ASPREE-XT Clinical Event Team and presented to adjudicators of the Depression EAC. Adjudicators are blinded to participant identity and original treatment arm for ASPREE. Cases are sent to two adjudicators and if there is discordance in the decision, the case will be taken to a meeting of the EAC that has to be unanimous in the final decision. Any case can be taken to a meeting of the EAC for discussion if an adjudicator needs to seek clarification in interpreting the notes or applying the decision rules.

Time-to-event for depression will be taken as the time between randomization and the date of the first elevated CES-D 10 score (for incident depression), or date of hospitalization (that is subsequently confirmed as a depression endpoint by the Depression EAC).

Depression and Dementia assessments

The CES-D 10 is also being administered in conjunction with the 3MS to ensure that a low score on the 3MS is not the result of depression. In the context of a participant's 3MS score declining to the dementia assessment trigger range (as described above), any participant also scoring 8 or more on the CES-D 10 will be referred to their usual physician for assessment and treatment, and will then be reassessed with the 3MS and CES-D 10 in ~3 months. If the CES-D 10 score has returned to below 8 at reassessment, or if the CES-D 10 was 8 or more at baseline, and the adjusted 3MS score at reassessment is still in the trigger range, a dementia assessment will proceed as usual. (Likewise, a dementia assessment will proceed if the past three CES-D 10 scores were all elevated). If the adjusted 3MS has returned to above the trigger at reassessment, the participant continues in the study without undergoing a dementia

assessment (and if the CES-D 10 remains 8 or more, the participant's usual physician is advised for further management).

DISABILITY (PERSISTENT PHYSICAL DISABILITY)

Definition and process for assessment/adjudication

Persistent loss of physical activities of daily living is defined as inability or severe difficulty with performance of one or more of the six Katz Activities of Daily Living (ADLs) [Katz, 1983] or, if the Katz ADL questions cannot be administered, eligibility/approval for admission to a nursing care facility because of a physical disability.

- The Katz ADLs include walking, bathing, dressing, transferring from a bed or chair, using the toilet, and eating.
- The following response options will be used: (1) no difficulty, (2) a little difficulty, (3) some difficulty, (4) a lot of difficulty, or (5) unable to perform. An additional question is asked for each Katz ADL about usually requiring assistance from another person.

For participants who are unable to answer due to illness, a proxy is permitted to answer these same questions on behalf of the participant. The instrument will be administered by interview at 6-monthly intervals, i.e. at the first ASPREE-XT study visit or first 6-month phone call (whichever is due first), and every six months thereafter. Persistent loss of the same Katz ADL was a primary end point for ASPREE, and remains a main outcome for ASPREE-XT.

In 2016, the ASPREE DSMB approved the expansion of the definition of persistent physical disability end point to include adjudicated eligibility for admission to a nursing care facility for physical disability in those cases where it was not possible to obtain Katz ADL information.

The Physical Disability EAC is responsible for determining if events should be considered a physical disability end point *in lieu* of information regarding the persistent loss of physical function as defined by a report of 'a lot of difficulty', 'unable to perform', or as a check, needing assistance with completing the ADL.

Source information from clinical case notes, nursing home facility records, and other medical records related to these events will be collected, sent to the ASPREE-XT Clinical Event Team and presented to adjudicators of the Physical Disability EAC. Adjudicators are blinded to participant identity and original treatment arm for ASPREE.

Time-to-event for the persistent Katz ADL loss will be taken as the first reported date of the ADL loss that is confirmed approximately 6 months later (+/- 1 month), or time to the date of admission to a nursing care facility for a physical disability.

DISABILITY-FREE SURVIVAL

Disability-free survival is a composite endpoint reached when the first event of death or dementia or persistent physical disability is reached.

Details of 'time-to-event' data capture for death, dementia and persistent physical disability (the composite endpoint that represents disability-free survival) are included above.

In summary, a dementia trigger occurs when there has been a reduction in 3MS (<78 or 10.15-point age and education level adjusted fall from baseline; administered annually), or a report of clinical diagnosis of dementia, or a report of a prescription of cholinesterase inhibitor for dementia (Australia only). The latter two triggers could occur at any annual visit or 6-month phone call (dementia diagnosis only). The event date for a dementia end point will be taken as the trigger date prompting a dementia assessment, which subsequently is confirmed as an end point by the Dementia EAC. The persistent physical disability end point will be taken as the first reported date of an Activities of Daily Living loss that is confirmed approximately 6 months later (+/- 1 month).

For the analysis of outcomes: a) All-cause mortality; b) Dementia; and c) Persistent Physical Disability, all participants experiencing the event will be included (not just the subset for whom the event was their first, thereby contributing to the composite ASPREE primary outcome of death or dementia or persistent physical disability – the primary endpoint for ASPREE).

MAJOR HEMORRHAGE or CLINICALLY SIGNIFICANT BLEEDING

Definition and processes for adjudication

Major hemorrhage includes:

a) hemorrhagic stroke, and

b) non-stroke clinically significant bleeding (CSB)

a) Hemorrhagic stroke definition and adjudication

Refer to the stroke section c) of Cardiovascular Disease, above.

b) Clinically significant bleeding (CSB) definition and adjudication

CSB is defined as non-stroke intracranial bleeding and extracranial bleeding at gastrointestinal or other sites that require transfusion, hospitalization for more than 24 hours, prolonged hospitalization by more than 24 hours with bleeding as the principal reason, surgery, or is fatal [Margolis *et al*. 2018].

The ASPREE-XT definition of CSB requires that bleeding is substantiated by the documentation of one of the following on the medical record:

- Observed bleeding (e.g. bleeding observed on gastroscope / cystoscope etc.)
- Reasonable report of symptoms of bleeding (e.g. melena or hematemesis)
- Medical, nursing or paramedical report
- Imaging evidence such as CT/MRI for intracerebral hemorrhage

Note: Low hemoglobin or drop in hemoglobin without one of the above does not satisfy the criteria of substantiated bleeding.

Specific decision rules developed by the CSB EAC include:

- If hospitalization criterion is to be utilized, bleeding must have been the principal reason for hospitalization, prolongation of hospitalization or surgery and must be substantiated.
- Additional adjudication will determine whether the event was spontaneous (e.g. bleeding esophageal varices or gastric ulcer) or induced (e.g. trauma).
- Elective in-patient surgical procedure (includes therapeutic endoscopic procedures) with prolonged stay, repeat surgery, or transfusion:- ‘Case does not meet CSB definition’
- Elective in-patient surgical procedure (includes therapeutic endoscopic procedures) readmitted after discharge primarily for bleeding:- ‘Case meets CSB definition’
- Elective out-patient procedure (includes therapeutic endoscopic procedures) readmitted, prolonged stay, repeat surgery, or transfusion:- ‘Case meets CSB definition’
- Non-elective inpatient procedure (includes therapeutic endoscopic procedures) readmitted, prolonged stay, repeat surgery, or transfusion:- ‘Case meets CSB definition’
- A positive fecal occult blood test is insufficient to substantiate observed CSB:- ‘Case does not meet CSB definition’

Source information from clinical case notes and hospital medical records related to these events will be collected, sent to the ASPREE-XT Clinical Event Team and presented to

adjudicators on the CSB EAC. Adjudicators are blinded to participant identity and to original treatment arm for ASPREE. Each blinded case will be sent to two adjudicators and if there is discordance in the outcome, the case will be sent to a third adjudicator for a decision. Any case can be taken to a meeting of the EAC for discussion if an adjudicator needs to seek clarification in interpreting the notes or applying the decision rules.

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APPENDIX 3. SAMPLE SIZE & STATISTICAL ANALYSIS PLAN

Sample Size

All living ASPREE participants, who have not withdrawn from ASPREE, are eligible for ASPREE-XT. In Australia, this number is expected to be approximately 14,000 people. In the US, this number is expected to be approximately 1,500 people.

Overview of the Statistical Analysis Plan for ASPREE-XT

Analyses for Aims 1, 2 and 3:

Aims 1-3 will undergo primary analyses according to intention-to-treat principles, i.e. according to the group to which participants were originally randomized and without reference to their actual compliance with assigned treatment or open-label aspirin either before or after the cessation date of the trial's intervention phase (12 June 2017).

Exploratory analyses will be undertaken that will account for actual use of aspirin. Specifically, we will analyze the effect of taking aspirin by using a rank-preserving structural failure time model with a switch time from aspirin use (whether randomized or open label) to no aspirin use, or a switch time from randomized placebo or no study drug use to open-label aspirin use. Endpoints for which legacy effects are hypothesized will also be analysed to estimate the complier-average causal effect of daily low-dose aspirin versus placebo in those individuals having the propensity to comply fully with protocol-defined durations of exposure.

As reported in the main ASPREE outcome publication in NEJM, randomization has adequately balanced baseline characteristics of participants in the two treatment groups [McNeil *et al*. 2018]. Hence unadjusted intention to treat analyses will be considered primary for Aims 1-3. Secondary analyses may include an adjustment for baseline (at time of randomization) characteristics that are thought to be strongly prognostic of outcome in this population (e.g. age, sex and comorbidities including cognitive function). For all endpoints the primary analysis will be a Cox proportional hazards regression model with randomized treatment group as the single covariate.

For disability-free survival and all-cause mortality, Kaplan-Meier estimates of cumulative incidence will be plotted for the two randomized groups. For all other outcomes in Table 3 above, estimates of cumulative incidence will be plotted for the two groups having been obtained from group-stratified models in which death (from causes other than any causes incorporated in the outcome of interest) will be considered competing risks.

For most outcomes it is unlikely that the effect of treatment will be constant over the full duration of analysis time, i.e. throughout the period of ASPREE (when participants may have had varying degrees of compliance) and the period of ASPREE-XT (when participants will no longer be on randomized treatment and may have used open-label aspirin for varying time periods). Hence a secondary set of analyses will compare outcomes between randomized groups using restricted mean survival time, RMST, at 10 years after randomization [Royston & Parmar, 2011]. The RMST will be estimated from Royston-Parmar models that allow for time-dependent effects of treatment by incorporating an interaction of treatment with a spline function for the cumulative hazard of events.

Additionally, for the endpoints of major hemorrhage and CVD for which it is envisaged that aspirin may have an early effect that reduces in strength during ASPREE-XT, a generalized ("combined") test of treatment effect, comparing RMST difference at 10 equally spaced intervals of time between 3 years post-randomization (approximately the minimum duration of

follow-up in ASPREE) and the time of the latest occurring event in ASPREE-XT (anticipated to be at approximately 13 years) will be used to test for the existence of a treatment effect at some point during that time interval while controlling for the multiplicity of the time points being employed [Royston & Parmar, 2016]. For endpoints such as cancer, for which it is envisaged that aspirin may have a different impact as a legacy effect following aspirin exposure, a landmark survival analysis will be undertaken with resetting of time zero to the landmark time in Cox proportional hazards regression. Two analyses will be undertaken, one restricted to participants still at risk of the endpoint at the 5 year post-randomization landmark time point and a second restricted to participants still at risk on the 12 June 2017 landmark ASPREE calendar time.

Analyses for Aims 1-3 will also be undertaken within sub-groups to examine for evidence of variation in the treatment effect. These sub-group analyses will be based on appropriate interaction terms in the relevant regression model for each outcome of interest (Cox proportional hazards regression model, Royston-Parmar parametric model, or landmark model). The p-values for these interaction terms will be used to test for heterogeneity of treatment effect of aspirin between sub-groups. There will not be any multiplicity adjustment when calculating p-values for sub-group analyses. Sub-group analyses will be labelled as exploratory in the reporting of longitudinal outcomes based on previous ASPREE treatment groups, and effect estimates and confidence intervals for each sub-group will be reported along with the p-value for the test of interaction. The sub-groups are defined using baseline (time of randomization) information, divided into pre-specified during ASPREE and new sub-groups, and include:

Pre-specified baseline sub-groups for ASPREE:

a) Males versus females

b) Age: (i) below and above study median (74 years) and (ii) in the categories 65-69, 70-74, 75-79, 80+ years

c) Country: U.S. versus Australia

d) Ethnicity: Whites in Australia, Whites in U.S., African Americans, Hispanics, Other (where 'other' will include Native Americans, Asians and Aboriginal Australians)

e) Diabetes: The presence or absence of diabetes as self-report, elevated fasting blood glucose [≥126 mg/dL (U.S.) or ≥7mmol/L (Australia)] or prescribed medication for diabetes

f) Hypertension: Hypertensives versus non-hypertensives. Hypertension is defined as those who are on treatment for high blood pressure or those with blood pressure recorded above 140/90 mmHg.

g) Smoking: Current versus Never or Former smokers

h) Prior aspirin use: self-reported regular use of aspirin immediately prior to baseline

i) Frailty: Frail versus Pre-frail versus Not frail. Adapted from Fried criteria for frailty, participants will be defined as *frail* if they satisfy at least three, and *pre-frail* if they satisfy one or two, of the following five criteria:

(i) BMI <20 kg/m^2,

(ii) Grip strength in the lowest 20% of participants by sex and Fried-defined sex-specific BMI categories,

(iii) At least one of the following conditions is present for 3 days or more during the last

week according to CES-D responses:

(a) I felt that everything I did was an effort

(b) I could not get going

(iv) Time to walk 3 meters (10 feet) is in the lowest 20% of participants by sex and Fried-defined sex-specific height categories,

(v) In the last 2 weeks, no walking outside the home, or walked outside home but longest amount of time walked without sitting down to rest was less than 10 minutes.

j) Personal history of cancer: History of any cancer at baseline other than non-melanoma skin cancer

k) Hypercholesterolemia (dyslipidemia): self-reported use of a statin at baseline or elevated cholesterol (either serum total cholesterol ≥240 mg/dL [≥6.2 mmol/L; for participants in the U.S.] or ≥212 mg/dL [≥5.5 mmol/L; for participants in Australia] or LDL>160 mg/dL [>4.1 mmol/L])

l) BMI: defined according to World Health Organization criteria as underweight (<20 kg/m^2), normal weight (20-24 kg/m^2), overweight (25-29 kg/m^2) or obese (≥30 kg/m^2)

m) CKD: defined as estimated glomerular filtration rate, eGFR, <60 ml/min/1.73m^2 or albumin to creatinine ratio ≥3mg/mmol or history of renal transplant or routine kidney dialysis.

Additional baseline sub-groups for ASPREE-XT:

n) Waist circumference: defined according to WHO sex-specific criteria as substantially increased disease risk at >88 cm for women and >102 cm for men.

o) Polypharmacy: defined as taking 5 or more medications.

Sensitivity analyses will be undertaken to examine reasons for loss to follow-up and to allow for possible informative censoring. In particular, loss to follow-up in the bridge period between ASPREE and ASPREE-XT will be examined for any impact on the assumption of uninformative censoring with an expectation that, since all deaths will be captured from publicly available records, this issue is anticipated to be of limited concern for the endpoint of all-cause mortality. Other sensitivity analyses will include the use of the Anderson-Gill model to undertake survival analyses that accommodate time to all events experienced by an individual participant meeting an endpoint definition.

Analyses for Aim 4

Aim 4 is broad: to determine "*Demographic, comorbid, genomic and environmental factors that contribute to the maintenance of cognition and other aspects of healthy aging in a highly phenotyped cohort of older adults*".

For ASPREE-XT study outcomes that are in the form of time-to-event, for example dementia incidence and all-cause mortality, groups of participants will generally be analyzed using Cox proportional hazards regression with adjustment for appropriate factors that may have confounding effects as hypothesized through the use of causal diagrams. If time-varying effects of an exposure are expected then the model will be generalized to accommodate this in a standard way. Likewise if time-varying exposures or confounders are of interest then these will be accommodated in a similar fashion. If time-dependent confounding is suspected then

approaches such as marginal structural models will be pursued. ASPREE-XT study outcomes that are measured on repeat study visits, for example, cognitive function and SF-12 quality of life, will generally be analyzed using mixed effect linear models with similar considerations for time-dependent features of the exposure-outcome relationship.

Beyond the generic approaches described above, the specific choice of any statistical analysis method for Aim 4 will be dictated by the specific aims of each area of research interest. It is anticipated that these analyses will need to overcome a number of potential biases to simple analysis approaches, for example attrition of individuals from the study cohort due to mortality, imperfect compliance of participants with randomized treatment during the intervention phase, self-selected exposure to LDA in post intervention phase, and the presence of multiple disease processes. The proposed methodological approaches to key areas of concern include:

Continuous biomarker tracking. We will analyze trajectories of cognitive function, changes in score-based measures of health such as frailty, and change of biomarkers such as eGFR over time. Observation of these patterns of change will be interrupted by mortality and change may be predictive of death [Brilleman *et al.* 2017]. Joint modeling of continuous measures and time-to-event will be adopted using standard joint modelling software [Rizopoulos, 2012]. Where more complex joint models are required, we will use software for multiple measures and possibly complex time to event models in R statistical software, which interfaces with the Bayesian model specification and estimation approaches offered by the software package Stan [Stan Development Team, 2018; Brilleman *et al*. 2018].

State transition models. Multistate time-to-event models will be employed to address the analysis of complex disease pathways such as for frailty and disability, and mild cognitive impairment and Alzheimer's disease, that consist of multiple discrete states in which both onset and transition between states are possible [Beyersmann *et al*. 2012]. The same modelling framework will be applied to cancer to disentangle transitions of study participants from being cancer-free to cancer incidence, metastasis, cancer mortality and death from other causes, as well as downstream transitions from incident cancer to metastasis and death (cancer-related or otherwise), and late-stage transitioning from metastasis to cancer-specific death and death from other causes.

Development of prognostic models specific to older people will be developed for the purpose of guiding treatment decision making and informing patients of likely prognoses; for example, for cognitive resilience, Alzheimer's disease, bleeding risk, resilience to frailty, and cardiovascular disease. The internal validation approach will be to use cross-validation, and measures of discrimination and calibration will be employed to summarize model performance. External validation opportunities will be actively sought and are viewed as being an important part of developing a new model.

Mechanism of treatment effects. Many research questions will require elucidation of whether exposure effects on intermediate/surrogate endpoints (such as tumor response or disease progression) are able to predict the effect on overall survival (or downstream clinical outcomes). Standard mediation analyses that dissect a total treatment effect into direct and indirect effects will be the starting point of these explorations [Baron & Kenny, 1986]. Dissection of an exposure's direct effect and an effect that is modulated through subsequent intermediate events will be undertaken using a time-varying covariate for the latter in extended Cox proportional hazards regression models or, if more complex confounding structures need to be considered, then through application of causal mediation approaches [VanderWeele, 2009]. Dissection of an exposure's direct effect and an effect that is modulated through altered *trajectory* of biomarker(s) will be achieved through use of joint models [Rizopoulos, 2012].

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APPENDIX 4. CONFLICT OF INTEREST

1. The ASPREE and ASPREE-XT projects are federally-funded studies from the National Institutes of Health. All recipients of federal funding are required to have their own Financial Conflict of Interest policies. This Conflict of Interest policy is in addition to any institutional Financial Conflict of Interest policies that the recipient adheres to as a recipient of federal funding.
2. This full policy is to be made public on our Website and in publications when possible.
3. The primary concerns are twofold. First, that the ASPREE-XT investigators maintain the internal integrity of the study by which we mean the confidence among ourselves (investigators and staff) as we develop and modify the detailed protocol, that advice is being given and decisions are being made in as unbiased and fully informed manner as possible. Second, that we maintain the external integrity of the study by which we mean the acceptance of our process and results as having met public standards of conduct.
4. To meet these goals we will obtain full disclosure by all of the key members of the study (as defined below in item 4) of their, and their immediate family's, financial relationships with all pharmaceutical and biomedical companies judged to have an active or potential interest in the conduct and outcome of the longitudinal ASPREE-XT study. These are to be reported annually on a standard form, each of which will be reviewed on at least an annual basis or more frequently if there is a significant change from the last report, by an Oversight Committee. The Oversight Committee will be comprised of the Chair of the Steering Committee and the Chair of the International Operations Committee. The information to be reported will not include specific dollar amounts, although the definitions below require that certain relationships be segregated by those above and below certain dollar thresholds.
5. All of the study PIs, Co-PIs, and the Steering Committee and its various subcommittees' members are covered by this policy.
6. A conflict of interest will not necessarily exclude any member of the study from participating in study discussions, unless required in individual cases by the Oversight Committee. However, full disclosure of all potential conflicts of interest will be made at Committee meetings to all attendees in an effective, but non-cumbersome manner. This includes the full Steering Committee as well as each of its sub-committees.
7. A significant financial conflict of interest, defined below, will cause a person to excuse himself or herself from voting on all issues related to the conflict. All such actions will be recorded and kept as part of the study record by the Oversight Committee.
8. All financially relevant relationships are to be reported. Only those relationships that are between the individual and the specific company (rather than between the individual's parent institution and the specific company, for example) present the potential for a significant financial conflict of interest, defined under paragraphs 10a and 10b below. Specifically, research funding for contracts or grants to the parent institution which provide support to the individual, his/her laboratory or his/her close scientific collaborators is not ordinarily judged to present the potential for a financial conflict of interest, although such awards are to be fully disclosed as a part of this policy.
9. Those financially relevant relationships that are to be reported include employment, consultancies, board memberships, honoraria, stock ownership or options, grants, contracts, patents received or pending, and royalties. The Oversight Committee will decide, with #9 below as a guideline, whether any of these and other relationships in each individual case is significant enough to warrant excuse from voting or discussions.

10. A significant financial relationship is defined to exist:

a) when the dollar amount awarded on an annual basis, or expected-to-be awarded on an annual basis, with regard to each related corporate relationship exceeds $10,000. The Oversight Committee may also judge lower dollar amounts as significant in specific/individual circumstances.

b) when there is any equity holding in a related company (excluding mutual funds and blind trusts). Again the Oversight Committee may decide in individual circumstances that the equity holdings are relatively minor enough to not present a real conflict of interest.

c) Significant financial relationships in existence since January, 2018 between ASPREE-XT investigators and all pharmaceutical and biomedical companies, judged to have an active or potential interest in the conduct and outcome of the longitudinal study, will be described in all study reports and publications. In addition, we will meet or exceed the reporting standards of the journals publishing our manuscripts.

APPENDIX 5. OSMB CHARTER

ASPirin in Reducing Events in the Elderly (ASPREE) Follow-up Phase

Observational Study Monitoring Board (OSMB) Charter

Introduction

A seven-member OSMB will act in an advisory capacity to the National Institute on Aging (NIA) to monitor data quality and evaluate the progress of the follow-up phase of the ASPREE study conducted by Dr. Anne Murray of the Berman Center for Outcomes and Clinical Research, Minneapolis, MN, Dr. John McNeil of Monash University, Melbourne, Australia and Dr Andy Chan of Harvard University, Boston MA. The follow-up phase started on June 13, 2017 after the ASPREE intervention phase ended. ASPREE intervention phase procedures (other than those related to the intervention) were maintained until December 2017. A new protocol for the remainder of the follow-up period (ASPREE-XT) was prepared for review by the OSMB.

Responsibilities of the OSMB

- Review and approve the IRB-approved ASPREE follow-up phase protocol and consent documents as well as the Standard Operating Procedures (SOP) prior to protocol initiation. Review and approve any changes to the protocol, consent document templates and SOP during the study.
- Monitor participant retention, protocol adherence, annual visit completion, study medication cessation, participant follow-up status, data completeness, outcome adjudication process and status of data cleaning. Advise NIA if corrective measures are needed.
- Monitor rates of important health events. If there are clinically important differences between study groups, advise NIA on the need for, timing, and mode of dissemination of these findings.
- Review proposed ancillary studies with regard to safety and impact on participant burden, and make recommendations on approval of these studies.
- Upon request by NIA, provide comments to NIA on analyses for major study outcome papers and drafts of these papers.

Communications

To mitigate any potential conflicts of interests, all communications between the investigators and the OSMB members on any issues related to the study shall be held through the NIA Project Officer (PO) or NIA Clinical Trials Operations Support Center (CTOSC) contractor. However, the investigators shall make presentations, discuss the reports, and ask and answer questions during the OSMB meetings and webinars/teleconferences.

Scheduling, Timing, and Conduct of Meetings

The OSMB meetings will be held either in person or via a webinar/teleconference at least two times a year at the call of the Chairperson and/or NIA PO. Meetings and webinars/teleconferences will be scheduled by the study staff or by NIA CTOSC. Four OSMB members will constitute a quorum. The NIA PO and/or NIA Project Scientist will be present at every meeting. Meetings will be closed to the public because discussions may address

confidential participant data. The OSMB meetings will consist of the open session and two optional sessions – Closed and Executive.

- **Open session** will be attended by the OSMB members, NIA, CTOSC and study staff. During the open session, information will be presented to the OSMB by the study and NIA staff, as appropriate, followed by general discussion.
- An optional **Closed session** could be called by the OSMB members or NIA staff prior to or during a meeting. This session will be attended by the OSMB members and NIA and CTOSC staff to discuss any confidential or sensitive issues.
- An optional **Executive session** could be called by any OSMB member prior to or during a meeting and only OSMB will attend this section.

Meeting Materials

There will be only the Open Session Report presented to the OSMB, which may direct additions and other modifications to the report on a one-time or continuing basis. The agenda and meeting materials will be provided to the OSMB by NIA PO or CTOSC not later than 10 calendar days prior to the meeting. Accordingly, the investigators shall submit the meeting materials to NIA PO or CTOSC at least 12 calendar days prior to the meeting.

Reports from the OSMB

A formal report containing the OSMB's recommendations will be prepared by NIA or CTOSC staff. Given confidentiality concerns, the report will include recommendations, but no details about the OSMB's discussion. The draft report will be sent to the OSMB members for review and approval. Once approved by a majority of the OSMB members, the NIA PO will forward the formal OSMB recommendations to the investigators and will indicate whether NIA concurs with the report.

If there are unresolved differences of opinion among OSMB members, reports from the OSMB may consist of majority and minority reports. In these cases, majority and minority are required to approve their respective portions of the report. The report may also include a dissenting opinion by an individual Board member, which will be included as an addendum.

Confidentiality

All materials, discussions and proceedings of the OSMB are completely confidential. Members and other participants in OSMB meetings are expected to maintain confidentiality. OSMB members will be required to sign a Non-Disclosure/Confidentiality Agreement and will be required to inform the NIA PO about any new conflicts of interest developing.

APPENDIX 6. CANCER TUMOR TISSUE BANKING

Funding

From 2013-2017, the National Cancer Institute in the US (one of the Institutes within the NIH) funded the ASPREE Cancer Endpoints Study (ACES) as a subsidiary of the main ASPREE study. ACES allows for the exploration of DNA related molecular mechanisms, along with other physiological mechanisms, of aspirin's protective effect against cancer and cancer-associated mortality and metastases, using tumor tissue. Additionally, the role of aspirin on different cancer subtypes can also be examined. A key component of this study is to establish a biologic specimen repository for tumor tissue from the ASPREE healthy aging population in the US and Australia, for future use by ASPREE, NIA and NCI investigators, and academicians from the broader research community. Participants in the ASPREE-XT longitudinal, observational, follow-up study will be asked to agree to allow ASPREE-XT to be provided with a small specimen of tumor tissue collected at the time of diagnosis or treatment, in those diagnosed with cancer.

In 2016, Australia was awarded funds from the National Health and Medical Research Council (NHMRC) through the competitive grants process to fund ongoing contact with ASPREE participants in Australia through 6-month phone calls, to track colorectal cancer events through electronic health records and to collect samples of colorectal cancer tissue. This funding is from 2017-2021.

In 2017, the NCI contributed funds to ASPREE-XT for all-cause solid tumor tissue to be collected across both countries until January 2019. The collection and banking of cancer tumor tissue will continue and extend to include all cancer subtypes, through the funding of an NIH grant through the National Institute on Aging's U19 funding mechanism. The continued collection of tumor tissue under the U19 grant will support this biobanking activity during the ASPREE-XT observational study.

At a time in the future and under separate application, the stored tumor tissue will be analyzed to address specific questions regarding the association of biomarkers and major health outcomes. Future applications for ethical approval will be made regarding projects that address disease outcomes of interest observed during the 5 year period of the study. The tumor tissue collection in both countries would improve our ability to measure the longitudinal effects of aspirin on incident and recurrent cancer and metastases, and in turn, potentially lead to the development of preventive and therapeutic targets for these outcomes.

Participant Consent: At the time of the ASPREE-XT consent, each ASPREE participant will be asked to consent to ASPREE-XT investigators obtaining a small sample of tumor tissue collected in the process of cancer diagnosis and/or surgical removal of tumor tissue. Consent will require the ticking of a box at the end of the consent form (see below).

'Cancer Biopsy

During the course of the study, some people may have biopsies taken for cancer diagnosis. We ask you for permission to access a small sample of this biopsy, if available. ASPREE-XT will not require you to undergo any additional biopsies or procedures for access to this sample. This tumor tissue may be used in future research studies to explore molecular mechanisms of aspirin's protective effect against cancer, however we cannot yet predict exactly what future research might be performed.

It is possible that the future research on your samples might be performed by other researchers

in Australia or overseas. In that case, some of your samples will be sent to those research laboratories. These samples will be labelled with a code and the scientists at the other laboratories will not be able to identify you from the code. In some cases, the samples we collect are destroyed during the process of testing. Future research on your samples will only be performed with the approval of a Human Research Ethics Committee. We are asking for your 'unspecified consent', which means that you agree to allow any type of research to be performed in the future on the samples we obtain for this project.

The purpose of storing your sample is to answer questions in the future, so we expect to keep your tumor sample indefinitely. You can have any sample we obtain removed from our collection and destroyed by contacting the researchers listed in this document in writing.
Please tick the box at the end of this consent form to indicate permission for this access.'

Tumor Tissue Processing, Shipping and Storage:

Australia: ASPREE-XT staff will follow the appropriate procedures for tissue procurement, as dictated by institution from which the tissue is to be sourced (hospital or registry). Procured tumor specimens will be sent to the ASPREE-XT Center or the Monash Public Health Biorepository, located at the Alfred Hospital, Melbourne, Australia for storage. Application for access to these samples for new analysis projects will be subject to the usual ASPREE (-XT) governance and review, also requiring ethics approval for each project. There is potential for shipment to a US-Based Tissue Procurement Facility (pending project approval and future funding). Upon arrival at the central Monash facility, the tumor tissue will be barcoded, and this code scanned and entered into the Laboratory Information Management System (LIMS) prior to storage. At the time of tumor tissue collection, all relevant information relating to the tissue (*e.g*. pathology reports, tissue handling and patient case reports) will be copied or scanned, and electronically entered into the ASPREE-XT database. A Tumor Tissue Biospecimen Information Sheet will be completed by the study staff member, identified by only the participant's ASPREE-XT study number and acrostic, and the information contained therein entered into the ASPREE Healthy Aging Biobank LIMS. All hard copy Tumor Tissue Biospecimen Information Sheets will be stored in a secure location at the ASPREE Healthy Aging Biobank.

US: At the time of tumor tissue collection, all relevant information relating to the tissue (e.g. pathology reports, tissue handling and patient case reports) will be copied or scanned, and electronically entered into the ASPREE-XT database. A Tumor Tissue Biospecimen Information Sheet will be completed by the study staff member, identified by only the participant's ASPREE-XT study number and acrostic, and the information contained therein entered into the database via the web interface. All hard copy Tumor Tissue Biospecimen Information Sheets will be stored in a secure location at the US-Based Tissue Procurement Facility details will be entered into the reporting section dedicated to participant cancer endpoints.

Saliva collection for DNA (Aus only):

Saliva collection can be done in the presence of a study staff member at either a study site, clinic or self-administered within the participant's home, if deemed more practical. When collected by study staff, a Saliva Biospecimen Information Sheet is completed. When collected by the participant, the participant is asked to complete and return the Saliva Biospecimen Information Sheet with the saliva sample, to the clinic site via pre-paid postage envelope. An instruction sheet will accompany this document, providing clear instructions and further explanations to the questions being asked. Study staff will enter data from the Saliva

Biospecimen Information Sheet to the ASPREE-XT database and mail both the saliva sample and the Saliva Biospecimen Information Sheet within one month or as soon as practical, to the US-Based Tissue Procurement Facility (US specimens). Upon receipt of the Saliva Biospecimen Information Sheet, the study staff will enter final details into the database, and file the original in the participant's file. The saliva sample will be coded with ASPREE-XT participant ID, entered into the database, and linked to the participants ASPREE-XT study number and acrostic. In Australia, during an ASPREE-XT visit, participants who have not provided a blood or saliva sample to the ASPREE Healthy Aging Biobank may be approached to provide a saliva sample with consent (PICF) through the ASPREE Healthy Aging Biobank.