External Assessment Report commissioned by the Evidence Synthesis Programme on behalf of the National Institute for Health and Care Excellence – Protocol

Title of Project:	Clopidogrel genotype testing after ischaemic stroke or transient ischaemic attack (DAP 65)
NIHR Reference No:	135620
Version Number:	2.0
Date:	25/8/2022
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Plain English Summary

What is the problem?

A stroke occurs when the supply of blood to the brain is cut off. Symptoms of stroke happen suddenly and vary depending on which part of the brain is affected. They usually include problems with movement, speech, vision and the face drooping on one side. A related condition, known as "transient ischaemic attack" (TIA), occurs when the blood supply to the brain is temporarily interrupted causing a mini-stroke. There are around 100 000 strokes every year in the UK and around 60 000 people will have a TIA.

People who have a stroke or TIA are at greater risk of having another stroke. To reduce the chances of this happening, doctors will often prescribe medication. The most common medication used to prevent further strokes is called "clopidogrel". However, clopidogrel does not work for everyone. One reason for this is having specific variations of a common gene called the CYP2C19 gene.

What are we trying to find out?

We want to know whether introducing genetic testing to identify variations in the CYP2C19 gene for people who have had a stroke or TIA will mean that more people can receive treatment that will work for them, reducing the risk of having another stroke. We also want to know whether introducing this type of testing is a good use of NHS money.

What are we going to do?

We are going to review the existing research, send out a survey to labs that do genetic tests, and develop economic (cost) models to answer the following questions:

1. Do people who have had a stroke or TIA, and who have specific variations in their CYP2C19 gene, have fewer strokes if they are given an alternative treatment to clopidogrel?
2. Which test should people have to find out if they have the variation in their CYP2C19 gene that means that clopidogrel may not work so well for them?
3. Is testing people for CYP2C19 gene variations and then giving them treatment based on which gene variations they have a cost-effective use of NHS money?

1 Decision problem

1.1 Population

The population of interest for this appraisal is people who have had non-cardioembolic ischaemic stroke, minor stroke or transient ischaemic attack (TIA) and for whom clopidogrel treatment is being considered. Approximately 100,000 strokes occur every year in the UK and between 46,000 and 65,000 people experience a TIA.(1) Around 85% of strokes are ischaemic, occurring when the supply of blood to a part of the brain is interrupted, usually by a blocked artery.(1) It has been suggested that a TIA is not a separate pathological entity, but exists on an ischaemic stroke spectrum, constituting the mildest form.(2) Symptoms of stroke often occur suddenly and vary depending on the part of the brain being compromised but tend to include issues with movement, speech, facial drooping and vision.

The median age for stroke in the UK is 77 years and a quarter of strokes in the UK happen in people of working age.(3) Lifestyle factors associated with stroke and TIA include smoking, alcohol and drug abuse, physical inactivity and poor diet. The presence of cardiovascular diseases and medical conditions including diabetes mellitus, atrial fibrillation, chronic kidney disease and migraine are also risk factors for stroke.(1) Other risk factors include the use of anticoagulation medication, previous stroke/TIA, family history of stroke, lower education and genetic or hereditary factors. Strokes are more common in people with African-Caribbean or South Asian background (Stroke Association; Kings Fund).(4, 5)

People who have experienced a stroke or TIA are at an increased risk of further occlusive vascular events (e.g. ischaemic stroke, transient ischaemic attack and myocardial infarction).(6) TIA precedes stroke in 15% of cases, providing a crucial opportunity to prevent more severe stroke.(7) Risk of stroke after TIA has been found to be approximately 8% at seven days, 11.5% at one month, 17.3% at three months. Risk of recurrent stroke after minor stroke has been suggested to be 11.5%, 15% and 18.5%, respectively.(8) NICE TA210 recommends the use of antiplatelet medications as a preventative treatment for people who have had an ischaemic stroke or TIA.(9) This is discussed further in section 2.4 and includes clopidogrel treatment.

1.2 Target condition: Clopidogrel resistance

Clopidogrel (Plavix, Sanofi-Aventis, Bristol-Myers Squibb) is an irreversible adenosine diphosphate (ADP)-receptor antagonist with antiplatelet properties. It is available as branded and generic preparations and has marketing authorisation for patients who have recently had an ischaemic stroke or TIA.(10) Clopidogrel is a prodrug, which needs to be converted (metabolised) into an active form by P450 CYP enzymes.(11) A substantial proportion of the population are less able to metabolise clopidogrel to its active form and so clopidogrel does not achieve its pharmacological effect, usually the result of genetic variants, mainly in the CYP2C19 gene. This is known as “clopidogrel resistance”. As well as the CYP2C19 gene, other factors that may cause or exacerbate clopidogrel resistance include: taking drugs such as omeprazole which compete for metabolism by the CYP450

system (12), and factors such as obesity, diabetes, and hypertension. Thus, both genetic and clinical factors need to be considered when determining whether an individual will respond to clopidogrel treatment.

1.2.1 Genetic basis of clopidogrel resistance

Cytochrome P450 2C19 is one of the main enzymes that metabolises clopidogrel to its active form. This enzyme is encoded by the CYP2C19 gene. CYP2C19 is one of many genes associated with clopidogrel response but it is widely recognised as being the most validated genetic determinant.(13) The CYP2C19 gene has multiple variant forms (alleles) which produce CYP2C19 enzymes. These alleles are given a star (*) number for identification. The Pharmacogene Variation Consortium (PharmVar) has outlined more than 35 star (*) allele haplotypes.(14) The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2C19 genotype and clopidogrel therapy notes that CYP2C19 allele *1 pertains to normal function, and that *2 and *3 are the most common alleles associated with loss of function. The frequency range of the *2 allele in all populations is between 10%-61% and the frequency of the *3 allele is between 0.04-15%respectively.(15) A systematic review found that people who carried one or two of these alleles had an increased risk of stroke and composite vascular events in contrast to noncarriers among patients with ischaemic stroke or TIA treated with clopidogrel.(16) Some alleles, in particular allele *17, are associated with increased function.(13)

A person's genotype is their unique sequence of DNA, whilst their phenotype is the observable expression of this genotype. A person's phenotype (in this case how they will respond to (metabolise) clopidogrel) can be predicted based on their allele function combinations. Generally, people with the genotype of two normal function alleles (e.g. CYP2C19*1/*1) have the phenotype of normal metabolisers. Intermediate metabolisers have one normal function allele and one loss of function allele (e.g. CYP2C19*1/*2). Poor metabolisers have two loss of function alleles (e.g. CYP2C19*2/*3). Rapid metabolisers have one normal and one increased function allele (e.g. CYP2C19*1/*17) and those with two increased function alleles (e.g. CYP2C19 *17/*17) are ultra-rapid metabolisers.(13)

There are significant ethnic variations in the incidence of the different CYP2C19 alleles. The most common loss of function allele, the *2 allele, is most common in people from an Asian background (~27%), then from an African or Caribbean background (~18%) and less common in those from a European family background (~15%).(15) The *3 loss of function allele, is most common in those with an Asian family background (~2-7%) but is much less common in other populations (<1%).

1.3 Intervention/Diagnostic Test

This review focuses on two categories of CYP2C19 genetic testing: point-of-care tests (POCT) and laboratory-based tests. POCT include any analytical test carried out by a healthcare professional outside of the laboratory, although it is also possible to install near patient testing equipment in local laboratories which may overcome challenges associated with

storage of reagents.(17) These tests have the potential to deliver results more quickly than standard laboratory-based tests. Laboratory-based tests are conducted by laboratory technicians in the laboratory. In the National Health Service (NHS), genomic testing is generally delivered by a network of 7 Genomic Laboratory Hubs. Testing for CYP2C19 is not currently included in the National Genomic Test Directory of tests commissioned by the NHS in England. Table 1 provides an overview of some of the available CYP2C19 genetic tests.

Table 1 Characteristics of CYP2C19 point of care and laboratory tests

Name of test	Type of test	General information	CYP2C19 alleles targeted	Time to run test
Genomadix Cube CYP2C19 system	Point of care	Intended to be used in conjunction with clinical judgement and routine monitoring to determine therapeutic strategy for drugs metabolized by the CYP2C19 enzyme. Test kit cartridges must be stored between −15°C and −80°C and used within 15 minutes of removal from the freezer. Results are stored locally on a laptop connected to the device and can be exported as a PDF.	*2, *3, *17	The test takes 1 hour to run for each cartridge.
GeneDrive CYP2C19 point of care test	Point of care	Used for qualitative in vitro molecular diagnostic tests. Test for CYP2C19 under development and likely to be available to NHS in early 2023. Results will be able to be transferred electronically to patient records by internet or through third-party middleware, or printed with an optional label printer.	*2, *3, *4, *8, *17, *35	Less than 1 hour to run for each cartridge.
Sanger CYP2C19 sequencing	Laboratory	Routine genomic testing approach used in all NHS genomic laboratory hubs. This	All alleles	Depends on sample numbers and number of

Name of test	Type of test	General information	CYP2C19 alleles targeted	Time to run test
		test sequences a single DNA fragment at a time.		alleles being tested for – more will mean longer turnaround times
Next-generation CYP2C19 gene sequencing	Laboratory	Sequences millions of short DNA sequences in parallel.	All alleles	Quicker turnaround for large sample numbers compared to Sanger sequencing.
Targeted CYP2C19 gene variant	Laboratory	Targeted genotyping assay amplifies and detects specific variants in target genomic DNA. Examples include: • PCR-based SNP genotyping assays using fluorescent reporter systems, such as TaqMan (ThermoFisher) • Other PCR-based genotyping panels that use proprietary detection methods, such as the xTAG CYP2C19 Kit v3 (Luminex) • Variant detection using mass spectrometry, such as MassARRAY (Agena Bioscience) • Loop-mediated isothermal amplification (LAMP), such as the LAMP human CYP2C19 mutation KIT (LaCAR MDx Technologies)	Potential to target all alleles but usually target specific alleles.	The methods of detection, equipment requirements and throughput capability vary between systems.

1.4 Place of the technology in the treatment pathway

Guidelines on appropriate antiplatelet therapy for the secondary prevention of stroke vary. The two main guidance documents of relevance are NICE guidance NG128 on stroke and TIA (3) and guidance from the Royal College of Physicians (RCP) on therapy for secondary prevention for people with stroke.(18)

Everyone with a suspected stroke should be admitted to a specialist acute stroke unit following assessment by first responders. NICE guidance NG128 states that within 24 hours of ischaemic stroke onset, daily aspirin 300mg should be offered unless the individual is intolerant to aspirin. (3) Aspirin should be continued until 2 weeks after stroke symptoms begin or until discharged. For patients with TIA, NICE guidance (TA210) recommends urgent treatment with modified-release dipyridamole in combination with aspirin in the first instance.(9)

For people with high-risk TIA (often defined as patients with an ABCD2 score of ≥ 4)(19) or minor stroke, dual anti-platelet therapy of aspirin and clopidogrel is often used in line with guidance from the European Stroke Organisation, beginning with 2 weeks acute dual therapy. (20) After 2 weeks of acute treatment, NICE guidance recommends long term antiplatelet treatment with clopidogrel monotherapy.(3) However, in practice patients are often given dual treatment with aspirin and clopidogrel before moving to longer term clopidogrel monotherapy. The recommended duration of dual therapy varies according to guidance from up to 21 days,(8) 21 to 90 days,(21) or up to 90 days.(22) This is consistent with the NICE clinical knowledge summary on secondary prevention following stroke and TIA, updated in 2022, which states that "dual therapy with aspirin plus clopidogrel (for up to 90 days) or aspirin plus ticagrelor (for 30 days) may be initiated in secondary care for some people (for example people at high risk of TIA, or those with intracranial stenosis) followed by antiplatelet monotherapy."(22) In those who are intolerant of aspirin, the RCP guidelines suggest clopidogrel could be considered as initial treatment.(18)

Currently, genetic testing for clopidogrel resistance is not routinely performed in the NHS before using clopidogrel in ischaemic stroke or TIA patients. If genetic testing to inform preventative treatment is introduced in the NHS in people with stroke it could take place in hospital before long-term anti-platelet treatment is started 2 weeks post-ischaemic stroke, or sooner in the case of TIA. People with an allele suggesting poor or intermediate metabolism of clopidogrel could be treated with an alternative to clopidogrel, while those without these alleles would receive standard clopidogrel treatment. Alternative treatments could include the following:

- Aspirin
- High-dose aspirin
- Aspirin combined with dipyridamole
- Clopidogrel dose escalation *(Unlicensed)*
- Ticagrelor in combination with aspirin *(Unlicensed)*

Ticagrelor does not have marketing authorisation in the UK for the secondary prevention after ischaemic stroke or TIA. However, we have heard from clinicians that it is sometimes used in high-risk patients, although it is not considered in those at high-risk of bleeding due to an elevated bleeding risk. There is a suspended NICE technology appraisal on ticagrelor for preventing stroke (GID-TA10663) after previous ischaemic stroke or high-risk TIA. This was suspended by the company on 11 May 2021, who also withdrew their application for

marketing authorisation for stroke to the EMA in December 2021.(23) Ticagrelor in combination with aspirin for up to 30 days is however included as a potential treatment for secondary prevention for some people (for example people at high risk of TIA, or those with intracranial stenosis) in the 2022 NICE clinical knowledge summary on secondary prevention following stroke and TIA.(22)

2 Aim and Objectives

The overall aim of this project is to summarise the evidence on the clinical- and cost-effectiveness of genetic testing to identify clopidogrel resistance in people with non-cardioembolic ischaemic stroke or TIA. We have defined the following objectives to address the overall aim:

Objective 1: Do people who have genetic testing for clopidogrel resistance, and who are treated based on these results, have a reduced risk of secondary vascular occlusive events compared to those who are not tested and are treated with clopidogrel following standard guidelines?

Objective 2: Do people who have loss of function alleles associated with clopidogrel resistance have a reduced risk of secondary vascular occlusive events if treated with alternative interventions compared to treatment with clopidogrel?

Objective 3: Do people who have loss of function alleles associated with clopidogrel resistance have an increased risk of secondary vascular occlusive events when treated with clopidogrel compared to patients without loss of functions alleles who are treated with clopidogrel?

Objective 4: What is the accuracy of point of care genotype tests for detecting variants associated with clopidogrel resistance?

Objective 5: What is the technical performance (other than accuracy) and cost of the different CYP2C19 genetic tests?

Objective 6: What is the cost-effectiveness of different POCT and laboratory based genetic tests for clopidogrel resistance compared with not testing for clopidogrel resistance?

3 Methods for assessing clinical effectiveness

A systematic review will be conducted to summarise the evidence on the clinical effectiveness of clopidogrel genotype testing after ischaemic stroke, including minor stroke and TIA. The systematic review will follow the principles outlined in the Centre for Reviews and Dissemination (CRD) guidance for undertaking reviews in health care, the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy and the NICE Health Technology Evaluations Manual.(24-26) The systematic review will be supplemented by a survey of manufacturers of POCT tests and genomic laboratory hubs to collect information on the technical performance of the different CYPC19 genetic tests (Objective 5; Section 3.5).

3.1 Inclusion and exclusion criteria

3.1.1 Objectives 1, 2 and 3

Inclusion criteria for objectives 1, 2 and 3 are summarised in Table 2 Inclusion Criteria for Objectives 1, 2. Studies that meet the following criteria will be eligible for inclusion:

Table 2 Inclusion Criteria for Objectives 1, 2 and 3

	Objective 1	**Objective 2**	**Objective 3**
Participants	Adults or children who have experienced an Ischaemic Stroke or TIA	Adults or children who have experienced an Ischaemic Stroke or TIA and who have one or two CYP2C19 loss of function alleles associated with under metabolism of clopidogrel (e.g. *2 or *3)	Adults or children who have had an ischaemic stroke or TIA who are treated with clopidogrel alone or in combination with a second antiplatelet drug.
Intervention/ exposure	Any of the CYP2C19 genotype tests listed followed by any alternative antiplatelet drug(s).	Any alternative antiplatelet drug(s).	Presence of one or two CYP2C19 loss of function alleles for metabolism of clopidogrel (e.g. *2 or *3)
Comparators	No testing; all patients treated with clopidogrel alone or in combination with a second antiplatelet drug	Clopidogrel alone or in combination with a second antiplatelet drug	No loss of function alleles
Outcomes	Incidence of secondary vascular occlusive events Adverse events (e.g. bleeding or headache) Mortality Time to starting antiplatelet treatment, or to change of antiplatelet treatment Impact of test result on decisions about care Health care resource use (e.g. Length of hospital stay) Quality of life Healthcare costs		

	Objective 1	Objective 2	Objective 3
Study design	Randomised controlled trials or cohort studies	Randomised controlled trials or cohort studies	Cohort studies

3.1.2 Objectives 4 and 5

Inclusion criteria for objectives 4 and 5 are summarised in Table 3 Inclusion criteria for objectives 4 and 5 . Additional data for objective 5, in particular for standard laboratory-based tests, will be identified through a survey of laboratories (section 3.5). Studies that meet the following criteria will be eligible for inclusion:

Table 3 Inclusion criteria for objectives 4 and 5

Participants	Adults or children who have experienced an Ischaemic Stroke or TIA. If insufficient studies are found in these populations then we will include studies in other populations; we do not anticipate that test accuracy is likely to differ substantially based on population.
Index test	Either of the following POCT: Genomadix cube CYP2C19 system Genedrive system CYP2C19 test
Target condition	Presence of at least one CYP2C19 loss of function allele
Reference standard	Any reported laboratory-based reference standard for CYP2C19
Outcomes	Data on sensitivity and specificity or sufficient data to construct a 2x2 table of test accuracy. Test failure rate; number of people with variant forms of CYP2C19 (and incidence of particular alleles); time to results; ease of use of test; cost of testing
Setting	Any setting
Study design	Any primary study.

3.2 Study identification

Studies will be identified using bibliographic and non-bibliographic search methods following the guidance of the NICE handbook.(26, 27) We will use two searches:

Search 1 will address objectives 1, 2 and 3, taking the following form: (search terms for Clopidogrel) AND (search terms for CYP2C19)

Search 2 will address objectives 4 and 5, taking the following form: (terms for point of care tests) AND (terms for CYP2C19 OR terms for Clopidogrel))

The MEDLINE search strategies are reported in Appendix 9.1 using a search narrative.(28) They were developed by one researcher (CC) and checked by another (ET) using the PRESS checklist.(29)

3.2.1 Bibliographic searching

We will search:

- MEDLINE (MEDALL) via Ovid;
- Embase via Ovid;
- The Cochrane Central Register of Controlled Trials (CENTRAL) via Wiley; and
- The Cumulative Index to Nursing and Allied Health Literature (CINAHL) via Ebsco Host.

3.2.2 Non-bibliographic search methods

We will also search the following trials registry resources:

- ClinicalTrials.gov via https://www.clinicaltrials.gov/
- WHO International Clinical Trials Registry Platform (ICTRP) via https://www.who.int/clinical-trials-registry-platform;

Studies fulfilling eligibility criteria at full-text will be forward citation searched using the Science Citations Index (Clarivate) and the reference lists will be checked through manual review. We will screen reference lists of any reviews (systematic or non-systematic) identified by our searches.

3.2.3 Managing the searches

Data will be exported to EndNote 20 for deduplication using the default deduplication settings. The searches will be reported according to PRISMA and PRISMA-S guidelines.(30, 31)

3.3 Review strategy

Two reviewers will independently screen titles and abstracts identified by the searches. Full copies of all reports considered potentially relevant will be obtained and two reviewers will independently assess these for inclusion. Any disagreements will be resolved by consensus or discussion with a third reviewer.

Data will be extracted using standardised data extraction forms developed in Microsoft Access. Data extraction forms will be piloted on a small sample of papers and adapted as necessary. Data will be extracted by one reviewer, and checked in detail by a second reviewer. Any disagreements will be resolved by consensus or discussion with a third reviewer.

3.3.1 Objectives 1, 2 and 3

Data will be extracted on the following: study design (RCT or cohort study), objective that study addresses, funding sources (public, industry, mixed), study location, participants (type of stroke, age, sex, ethnicity), inclusion criteria, genetic test details, interventions (e.g. clopidogrel, alternative anti-platelet drug), and incidence of secondary vascular occlusive events (number in intervention/exposed group and number in control group). Data will also be extracted on the following secondary outcomes: adverse events (e.g. bleeding or headache), mortality, time to starting antiplatelet treatment, or to change of antiplatelet treatment, impact of test result on decisions about care, health care resource use (e.g. Length of hospital stay), quality of life and healthcare costs.

Dichotomous data will be extracted as number of patients with events and/or number of events and total number of patients in each treatment arm. For categorical data, we will extract details on the categories assessed, the total number of patients in each treatment arm and the number of patients in each outcome category. For continuous data we will extract means/medians together with ranges, standard deviations (SD), standard errors (SE) and/or confidence intervals (CIs) for the outcome at baseline, follow-up and for change from baseline in each treatment group. For all types of data, summary effect estimates together with 95% CIs and p-values for comparisons between groups together with details on the methods of analysis, any variables controlled for in the analysis and the test statistic will be extracted.

3.3.2 Objectives 4 and 5

Data will be extracted on the following: study design, funding sources (public, industry, mixed), study location, participants (indication for testing, age, sex, ethnicity), inclusion criteria, POCT genetic test details, reference standard details, and accuracy data. Where reported, data will also be extracted on the following secondary outcomes: test failure rate; number of people with variant forms of CYP2C19 (and incidence of particular alleles); time to results; ease of use of test; cost of testing.

Accuracy data will be extracted as 2x2 tables comparing the POCT with a laboratory reference standard. Where 2x2 data are not available, data will be extracted on any reported estimates of accuracy (e.g. sensitivity, specificity, area under the receiver operating characteristic curve (AUC ROC)). Each individual will have two alleles – one or both of these may be associated with loss of function. As described in Section 1.2.1, some alleles are associated with over-metabolism rather than poor metabolism (e.g. *17). As no difference in treatment is recommended in people who are over-metabolisers, these alleles will be grouped with those that are associated with normal function. This effectively gives three potential categories for each individual:

- Two loss of function alleles (e.g. *2/*2 or *3/*3 or *3/*2)
- One loss of function allele (e.g. *2/*1, *3/*1, *3/17, or *2/*17)
- Normal function (e.g. *1/*1 or *1/*17)

These categories will be dichotomised into alleles that encode for normal function and those that are non-functional. A "positive" test result (non-functional) will be the presence of at least one loss of function allele. A positive reference standard will be as reported in the study - either detection of any loss of allele function, or detection of those alleles that are detectable by the POCT evaluated. If data are reported for both possible reference standards then data will be extracted for both of these.

Where multiple sets of 2x2 data are reported in a single study, for example for different tests, thresholds, or alleles, all data will be extracted.

3.4 Quality assessment strategy

The methodological quality of included RCTs will be assessed using the updated Cochrane Risk of Bias Tool (ROB 2.0).(32) Cohort studies will be assessed using the ROBINS-I tool.(33) Diagnostic accuracy studies will be assessed for methodological quality using QUADAS-2.(34) Quality assessment will be undertaken by one reviewer and checked by a second reviewer. Any disagreements will be resolved by consensus or discussion with a third reviewer.

3.5 Web-based survey

We will conduct a web-based survey to gather data on the technical performance characteristics of CYP2C19 genetic tests (objective 5). The survey will be sent to the manufacturers of the two POCT tests and to all 7 genomic laboratory hubs who are responsible for delivering genomic testing in the NHS in England. The survey will collect information on:

- Preferred test platform for running CYP2C19
- Time to results
- Time/resources to perform test, including whether a faster turnaround is possible with additional resources, and what these might be
- Can test be performed in local testing laboratories
- Logistics
- Ease of use
- Test failure rate
- Cost of test
- Facilitators and barriers to implementing testing, and what platform would be most likely to be implemented
- How feasible would it be to install POCT tests in local laboratories
- Testing capacity
- How test would be affected by testing for all LOF alleles compared to only testing for *2 or *3 alleles.

3.6 Synthesis methods

For each objective, a narrative summary of all of the included studies will be presented. This will include a summary of the study characteristics (e.g. study designs, population sizes,

geographical location, year, baseline population characteristics, CYPC19 genetic tests evaluated, and outcomes reported) and of study quality. This will include the identification of any potential sources of bias or factors that may limit the generalisability of the findings.

3.6.1 Objectives 1, 2 and 3

If sufficient data are available, meta-analysis will be carried out to generate summary effect estimates for each objective. Where observational (cohort) studies are synthesised, estimates that have been adjusted for potential confounders will be used where available. Random effects meta-analysis will be performed where sufficient studies are available to enable a between-studies heterogeneity parameter (tau) to be estimated. A restricted maximum likelihood (REML) approach will be used to estimate tau.(35) Heterogeneity and inconsistency across studies will be quantified using the tau and I^2 statistics. (36) Fixed effect meta-analyses will be performed as sensitivity analyses, or as the sole analyses if insufficient data are available to estimate tau.

If sufficient data are available, we will explore potential heterogeneity in intervention effects using meta-regression and/or subgroup analyses. Variables that may be investigated as possible sources of heterogeneity include patient demographics (age, gender, ethnicity), severity of stroke (TIA, moderate or severe ischaemic stroke), whether clopidogrel was started earlier than 2 weeks after symptom onset, type of genetic test (POCT vs laboratory), loss of function alleles targeted, overall risk of bias and individual risk of bias domains.

In Objective 2, if sufficient data are available to compare more than one alternative treatment to clopidogrel, then a network meta-analysis (37) will be conducted to make comparisons across all treatment options for which there is a connected network of evidence.

3.6.2 Objective 4

Estimates of sensitivity and specificity of the POCTs will be calculated from each set of 2 x 2 data, under the assumption that the laboratory reference standards have correctly categorised all study participants. Bivariate random effects meta-analysis of sensitivity and specificity will be performed, with binomial likelihoods.(38, 39) Analyses will be stratified according to POCT test. Summary estimates of sensitivity and specificity together with 95% confidence intervals (CIs) will be calculated. Coupled forest plots of sensitivity and specificity will be used to display results from individual studies, to allow visual assessment of heterogeneity. Study-level and pooled results will also be plotted in Receiver Operating Characteristic (ROC) space, with 95% confidence ellipses around pooled estimates representing the joint uncertainty in sensitivity and specificity. If sufficient data are available we will investigate the following variables as possible sources of heterogeneity: patient demographics (age, gender, ethnicity), indication, operator characteristic (trained laboratory scientist vs other), loss of function alleles targeted, overall risk of bias and individual risk of bias domains.

3.6.3 Objective 5

We do not anticipate having sufficient data to carry out a meta-analysis for the secondary outcomes that address objective 5. We will provide a narrative summary of results from these studies which will be presented together with the results of the web-based survey (section 3.5).

Recommendations for further research will be made based on any gaps in the evidence or methodological limitations of the existing evidence base.

4 Methods for synthesising of cost effectiveness

4.1 Identifying and systematically reviewing published cost-effectiveness studies

We will conduct a systematic review to identify previous studies on the cost-effectiveness of CYP2C19 genetic tests for guiding treatment in non-cardioembolic ischaemic stroke and TIA patients. We will search the following databases, applying an economic search filter where possible:

- MEDLINE (MEDALL) via Ovid;
- Embase via Ovid;
- EconLit via Ovid;
- Centre for Reviews and Dissemination (HTA and NHS EED)
- Tufts CEA Registry

We will also include any relevant papers on cost-effectiveness identified in the clinical effectiveness reviews, search citations in relevant publications that we identify, and ask experts in the field.

We will assess the quality of the included cost-effectiveness studies using the Drummond checklist.(40) If no relevant models for CYP2C19 genetic testing are identified we will also search for economic models of treatments for ischaemic stroke and TIA.

We will run additional targeted searches to identify inputs to the economic model as required. For example, we envisage that this will include searching for previous network meta-analyses of antiplatelet treatments in general non-cardioembolic ischaemic stroke and TIA populations, in anticipation that there will be insufficient evidence on a CYP2C19 clopidogrel-resistant subgroup population for all alternative antiplatelet treatment options available.

4.2 Evaluation of costs, quality of life and cost-effectiveness

A decision-analytic model will be developed to estimate the incremental costs and quality-adjusted life years (QALYs) for CYP2C19 genetic testing for clopidogrel resistance in patients who have had a non-cardioembolic ischaemic stroke or TIA, compared with no genetic

testing. Two separate models will be developed: a model for patients who have had a non-minor ischaemic stroke; and a model for patients who have had a TIA or minor stroke. Results will be presented for the two sub-populations separately and also for the full population using a population average approach using the proportions in each sub-population (non-minor ischaemic stroke and TIA or minor stroke). Scenario analyses will be conducted for (i) populations with high prevalence of clopidogrel resistance and (ii) for children (if sufficient evidence is available).

4.2.1 Genetic testing and treatment strategies

The strategies that will be compared in the model comprise antiplatelet therapy with or without various pharmacogenomic tests. We will include POCT CYP2C19 genetic tests identified in the clinical effectiveness review (Section 3.1.1) and a single laboratory-based CYP2C19 genetic test chosen to be representative of how laboratory-based tests are likely to be used in practice (based on our survey of genomic laboratory hubs (Objective 5, Section 3.5)). We will vary the cost of the lab-based test in scenario analyses (see section 4.2.4).

For patients whose test indicates clopidogrel resistance, we will assume their treatment will switch to dipyridamole with aspirin as recommended by NICE guidance (22) with model parameters based on efficacy estimates from the clinical effectiveness review (Sections 3.1.1 and 3.6.1). If there is insufficient evidence to model outcomes for clopidogrel resistant patients on dipyridamole with aspirin, we will model an alternative antiplatelet therapy based on data availability and consultation with clinical experts. Other possible alternative antiplatelet therapies include:

- aspirin (with or without dose escalation)
- higher dose clopidogrel
- ticagrelor with aspirin

4.2.2 Model structure

The model structure will be developed to capture the short- and long-term costs and benefits of CYP2C19 genetic testing and will be informed by the findings of our review of cost-effectiveness studies and discussions with our clinical advisors. We envisage that there will be a short-term part of the model where initial therapy with aspirin occurs (or dual therapy immediately for TIA patients), and a longer-term part of the model where patients are taking long-term monotherapy for secondary prevention of vascular occlusive events (see Appendix 9.2 for a draft model structure). The short-term part of the model will have a decision tree structure to capture the diagnostic test accuracy of the POCT tests (assuming that the laboratory-based test is a gold standard test), and the impact of the time until results are available from the laboratory-based test which determines when an alternative treatment option can begin for patients identified as clopidogrel resistant. The model will assume results are available immediately after administering a POCT test but there will be a time lag until laboratory test results are processed. It will also capture the elevated risk of a subsequent stroke in the short-term following an event, which is particularly relevant for patients who have had a TIA. It is anticipated that the long-term part of the model will be a

Markov state-transition model including future strokes, intracerebral haemorrhage, and death. We anticipate that the model structure will be similar in the non-minor ischaemic stroke and minor stroke/TIA groups. However, as the treatment pathways, costs transition probabilities will vary, we plan to estimate cost-effectiveness separately for these two sub-populations.

An NHS and personal social services (PSS) perspective will be taken with a life-time horizon where costs and QALYs are discounted at an annual rate of 3.5%. The model will include all relevant health effects, including patients and other relevant people (such as carers).

Probabilistic sensitivity analysis where parameter uncertainty is captured with probability distributions and simulation will be used to estimate incremental cost-effectiveness ratios and expected net benefits at commonly used NICE willingness to pay thresholds. Uncertainty will be presented using cost-effectiveness planes and cost-effectiveness acceptability frontiers. One way sensitivity analyses will be performed for all key parameters, including all parameters based on expert opinion.

4.2.3 Model inputs

Model inputs will be derived from the clinical and cost-effectiveness reviews where possible, supplemented by targeted literature searches. Where there is insufficient evidence available we will base parameters on expert opinion and conduct scenario analyses to explore the impact of these assumptions on the results.

4.2.4 Scenario analyses

Scenario analyses will be conducted to explore the sensitivity of results to key model assumptions. Prevalence of clopidogrel resistance varies across populations, and it is to be expected that CYP2C19 genetic testing is more likely to be cost-effective in high prevalence populations. We will therefore a conduct scenario analysis for a population with high prevalence of clopidogrel resistance.

Some non-minor non-cardioembolic ischaemic stroke patients may start clopidogrel prior to 2 weeks (e.g. because they are already taking aspirin or they have been discharged early from hospital). We will run a scenario analysis where clopidogrel is started immediately in a non-minor non-cardioembolic ischaemic stroke population.

We envisage that the majority of the evidence will be in adult populations, however clopidogrel is sometimes used in children and young people. If sufficient evidence is identified for children who have had a non-minor non-cardioembolic ischaemic stroke or a TIA / minor stroke where the underlying cause and aetiology are aligned with our models, we will run scenario analyses using the same model structures with the model inputs adapted to be relevant to children.

We will run the model for an average age of the initial stroke event in line with the studies of clinical effectiveness identified in our review and will assess sensitivity of the results to changes in the average age in scenario analyses. In addition, scenario analyses will be conducted exploring the impact of variation in the time to receive results from laboratory tests as well as the impact of variation in the costs of laboratory tests by centre or region.

4.2.5 Health outcomes

Utility values associated with different severities of strokes, intracerebral bleeds, and other cardiovascular events will be estimated based on our reviews of previous cost-effectiveness models and targeted literature searches. These will be combined with state transition probabilities in the economic model to estimate QALYs.

4.2.6 Costs

Costs of the different genetic tests, treatments, and resource use associated with stroke, intracerebral bleed, and other cardiovascular events from routine NHS sources (NHS reference costs, Personal Social Services Research Unit (PSSRU), British National Formulary (BNF)), our reviews of previous cost-effectiveness models and targeted literature searches, and through discussions with the manufacturers and clinical advisors.

5 Handling information from the companies

All data submitted by the manufacturers/sponsors will be considered if received by the EAG no later than 14/11/2022. Data arriving after this date will not be considered. If the data meet the inclusion criteria for the review they will be extracted and quality assessed in accordance with the procedures outlined in this protocol.

Any 'commercial in confidence' data provided by manufacturers, and specified as such, will be highlighted in blue and underlined in the assessment report (followed by company name in parentheses). Any 'academic in confidence' data provided by manufacturers, and specified as such, will be highlighted in yellow and underlined in the assessment report. Any confidential data used in the cost-effectiveness models will also be highlighted. If confidential information is included in economic models then a version using dummy data or publicly available data in place of confidential data will be provided.

6 Competing interests of authors

None of the authors have any competing interests.

7 Timetable/milestones

Milestone	Date to be completed
Draft protocol	21/7/2022
Final protocol	16/8/2022
Progress report	14/11/2022

Draft assessment report	23/1/2023
Final assessment report	20/2/2023

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9 Appendices

9.1 Clinical effectiveness searches

We will use two searches to address the research objectives. The searches are not limited by date of publication or language.

9.1.1 Draft Medline Search 1: Objectives 1, 2 and 3

Database: MEDLINE (MEDALL)
Host: Ovid
Data parameters: 1946 to present
Date of search: 29 July 2022

Search syntax	Contextual detail
1 Clopidogrel/ (9880) 2 (clopidogrel* or clopidogrelum or M-clopidogrel or Nra-clopidogrel or grepid or iscover or duoplavin* or plavi* or Plavix* or zyllt* or "R 130964" or "R-130964" or R130964 or "SR 25990" or "SR-25990" or SR25990 or A74586SNO7 or "113665-84-2").ti,ab,kw,kf. (14144) 3 1 or 2 (16043)	Search for Clopidogrel Lines 1 or 2 search for Clopidogrel. Line 1 focuses on the controlled indexing term for Clopidogrel. The / indicates that this is a controlled indexing term. The free-text terms search in the following fields: * ti = title; * ab = abstract; * kw = author keyword; * kf = key field; and; * ot = original title. The structure in line 2 is the prevailing name of the intervention (clopidogrel), followed by alternate brand names or synonyms (e.g., duoplavin, plavix, zyllt) ATC codes or UNII code (A74586SNO7), and the CAS registry number ("113665-84-2").
4 Cytochrome P-450 CYP2C19/ (3309) 5 (CYP2C19* or cypiic19* or "Cytochrome P-450").ti,ab,kw,kf. (20434)	CYP2C19 Genotype We have truncated CYP2C19 (using the * marker) to identify CYP2C19*2, CYP2C19*3, and CYP2C9*17, and other alleles.
6 3 and 5 (1182)	Line 6 combines the search: Line 3 – terms for Clopidogrel. AND Line 6 – terms for CYP2C19

9.1.2 Draft Medline Search 2: Objective 4 and 5

Database: MEDLINE (MEDALL)
Hots: Ovid
Data parameters: 1946 to present
Date of search: 29 July 2022

Search syntax	Contextual detail
1 Point-of-Care Testing/ (3652) 2 ((Point of Care adj2 test*) or POCT).ti,ab,kf,kw. (9296) 3 (Genomadix* or GeneDrive or Spartan).ti,ab,hw,kf,kw. (340) 4 1 or 2 or 3 (11544)	Line 1 focuses on the controlled indexing term for Point of Care Testing. The / indicates that this is a controlled indexing term. The free-text terms (Line 2 or Line 3) search in the following fields: * ti = title; * ab = abstract; * kw = author keyword; * kf = key field; and; * hw = heading word.
5 Cytochrome P-450 CYP2C19/ (3312) 6 (CYP2C19* or cypiic19* or "Cytochrome P-450").ti,ab,kw,kf. (20353) 7 5 or 6 (20901)	Terms for CYP2C19 The free-text terms are truncated using the * marker. Truncation ensures that the root word and other possible variations are identified and returned by the search. We have truncated CYP2C19 (using the * marker again) to identify CYP2C19*2, CYP2C19*3, and CYP2C9*17, and other alleles.
8 Clopidogrel/ (9890) 9 (clopidogrel* or clopidogrelum or M-clopidogrel or Nra-clopidogrel or grepid or iscover or duoplavin* or plavi* or Plavix* or zyllt* or "R 130964" or "R-130964" or R130964 or "SR 25990" or "SR-25990" or SR25990 or A74586SNO7 or "113665-84-2").ti,ab,kw,kf. (13984)	Terms for Clopidogrel

10 11	8 or 9 (15885) 4 and (7 or 10) (92)	Line 11 combines terms for Point of Care testing AND terms for CYP2C19 OR terms for Clopidogrel.

9.2 Draft Model Structure

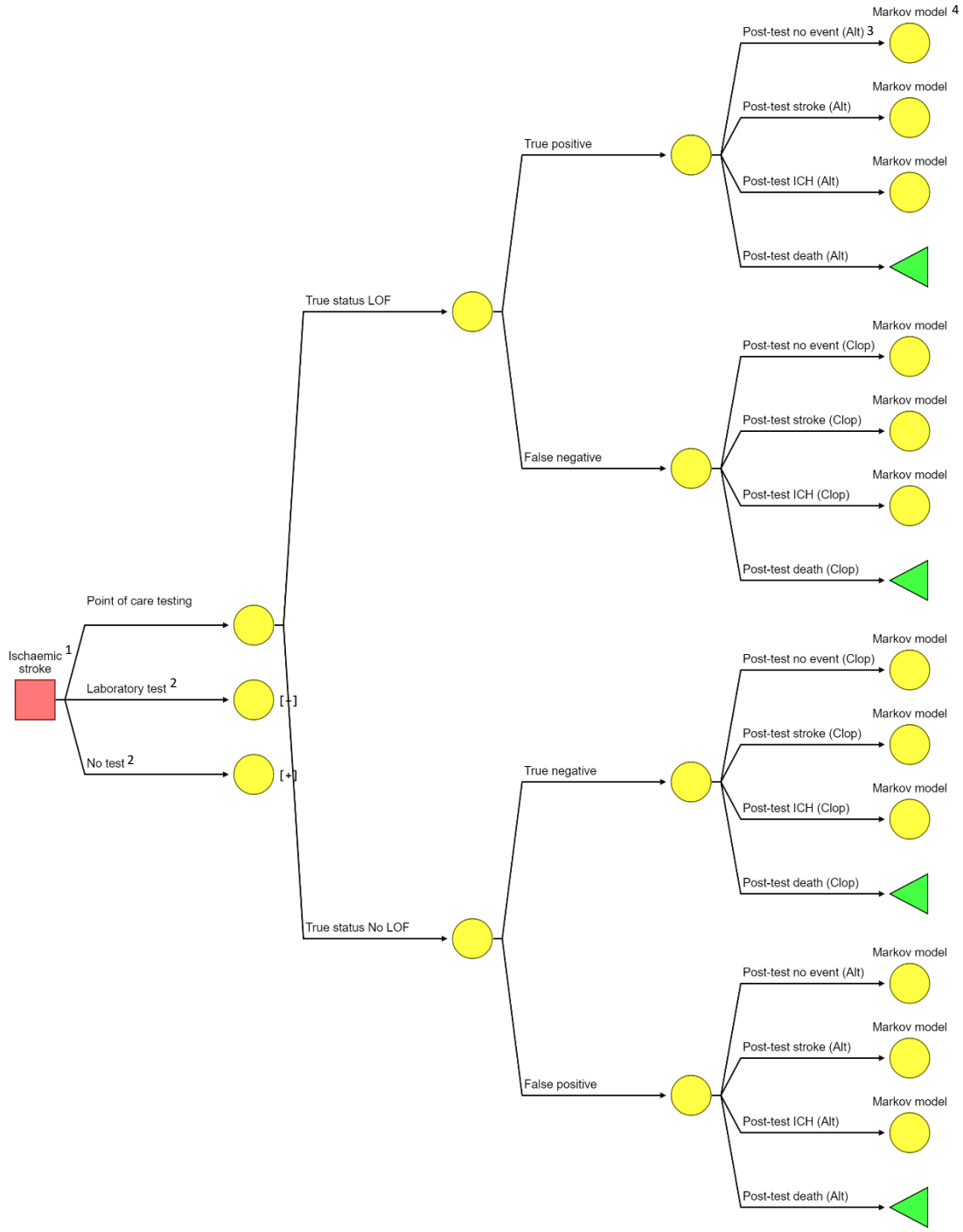

Figure 1 Point of care testing branch of the decision tree

[1] The timing of Clopidogrel treatment will depend on the indication. Those patients who have had a transient ischaemic attack/minor stroke may begin dual Clopidogrel-Aspirin treatment immediately. Those patients who have had a major stroke may initiate with 2 weeks of Aspirin monotherapy before Clopidogrel treatment

[2] Laboratory test and no test branches of the decision tree are outlined in Figure 2 and Figure 3

[3] Alternative treatments will include the anti-platelet comparator regimens. Alternatives outlined in the scope include: Aspirin monotherapy, high dose Aspirin monotherapy Aspirin combined with Dipyridamole, Aspirin combined with Ticagrelor

[4] Long-term patient outcomes will be modelled using a Markov structure outlined in Figure 4

Abbreviations: Clop, antiplatelet treatment regimen including clopidogrel; Alt, anti-platelet treatment regimen using alternative antiplatelet instead of clopidogrel; ICH, intracerebral haemorrhage; LOF, loss of function

Figure 2 Laboratory testing branch of the decision tree

[1] The timing of Clopidogrel treatment will depend on the indication. Those patients who have had a transient ischaemic attack/minor stroke may begin dual Clopidogrel-Aspirin treatment immediately. Those patients who have had a major stroke may initiate with 2 weeks of Aspirin monotherapy before Clopidogrel treatment

[2] Point of care test and no test branches of the decision tree are outlined in Figure 1 and Figure 3

[3] Alternative treatments will include the anti-platelet comparator regimens. Alternatives outlined in the scope include: Aspirin monotherapy, high dose Aspirin monotherapy Aspirin combined with Dipyridamole, Aspirin combined with Ticagrelor

[4] Long-term patient outcomes will be modelled using a Markov structure outlined in Figure 4

Abbreviations: Clop, antiplatelet treatment regimen including clopidogrel; Alt, anti-platelet treatment regimen using alternative antiplatelet instead of clopidogrel; ICH, intracerebral haemorrhage; LOF, loss of function

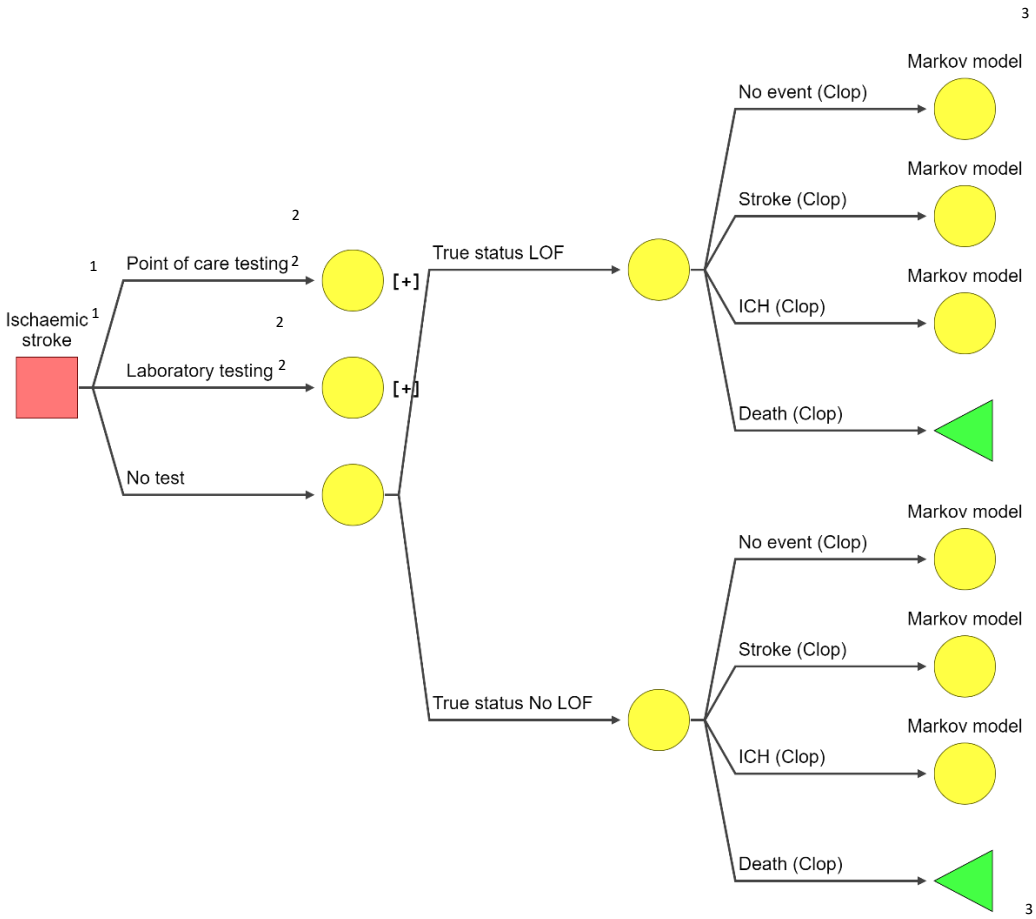

Figure 3 No test branch of the decision tree

[1] The timing of Clopidogrel treatment will depend on the indication. Those patients who have had a transient ischaemic attack/minor stroke may begin dual Clopidogrel-Aspirin treatment immediately. Those patients who have had a major stroke may initiate with 2 weeks of Aspirin monotherapy before Clopidogrel treatment

[2] Point of care and laboratory test branches of the decision tree are outlined in Figure 1 and Figure 2

[3] Long-term patient outcomes will be modelled using a Markov structure outlined in Figure 4

Abbreviations: Clop, antiplatelet treatment regimen including clopidogrel; ICH, intracerebral haemorrhage; LOF, loss of function

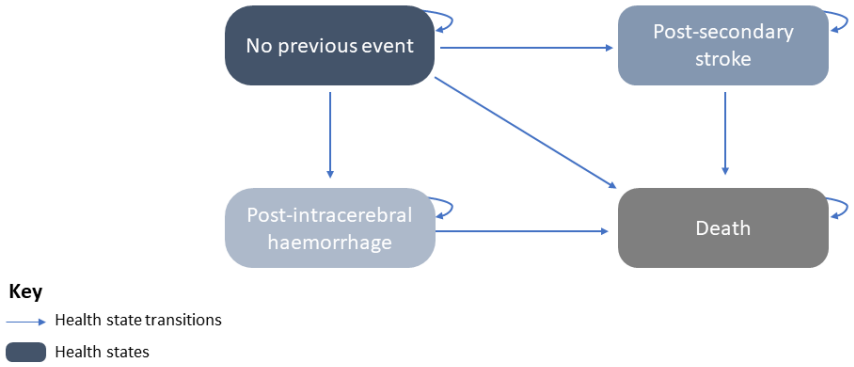

Figure 4 Long-term Markov model structure[1,2]

[1] Long-term patient outcomes will be modelled using a Markov model structure. Patients in the cohort will be assigned health states dependent on outcomes from the initial decision tree outlined in Figure 1, Figure 2, and Figure 3

[2] The potential long-term patient outcomes will not change based on treatment type, but the transition probabilities between health states may