Efficacy of Nitric Oxide in Stroke-2 (ENOS-2)

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SYNOPSIS

Title	Efficacy of Nitric Oxide in Stroke-2 (ENOS-2)
Acronym	ENOS-2
Short title	Efficacy of Nitric Oxide in Stroke-2 (ENOS-2)
Chief Investigator	Prof Philip Bath Deputy Chief Investigator: Prof Nikola Sprigg
Objectives	To assess the feasibility of recruitment and safety of transdermal glyceryl trinitrate (GTN) versus sham applied between 3 and 5 hours of stroke to inform a definitive trial.
Trial Configuration	Prospective randomised single-blinded masked-endpointphase IIb trial.
Setting	Adults with hyperacute stroke presenting at emergency departments and hyperacute stroke units in England.
Sample size estimate	This is a feasibility study so there is no formal sample size calculation. (But recruitment of 100 participants would allow an adjusted common odds ratio, acOR 0.51 to be detected, assuming mRS distribution as in [1]; alpha 0.05, power 0.80.) It is likely that a large definitive trial would be feasible if at least 70 participants were recruited into this study. Lower recruitment would not preclude progression if there was some evidence that the barriers to recruitment identified could be overcome.
Number of participants	• 100 with ischaemic stroke (50 randomised to GTN, 50 randomised to sham) • 20 with intracerebral haemorrhage (for safety) (10 randomised to GTN, 10 randomised to sham)

Eligibility criteria	Inclusion: 120 adults (≥18 years) with presentation compatible with stroke) Treatment 3-5 hours post ictus (for patients with wake-up stroke, treatment no more than 5 hours after patient awakens) One or more of the following symptoms present at time of enrolment: Dysphasia, neglect (NIHSS 1-2), hemianopia (NIHSS 1-3), or limb weakness (NIHSS on affected arm and/or leg 1-4) Systolic BP (≥120 mmHg) If a CT/MR scan has already been performed, then it shows acute intracerebral haemorrhage or ischaemic stroke, or is normal. Waiver of consent for treatment to ensure GTN given in 3-5-hour time-window (and thrombolysis not delayed if ischaemic stroke). Exclusion: mRS ≥4 Glucose (BM stix or equivalent) <3 mmol/l Glasgow coma scale ≤8 Witnessed seizure at presentation Known life expectancy <6 months. Patient presenting with sensory symptoms only Known stroke mimic, aneurysmal subarachnoid haemorrhage, or haemorrhage due to venous thrombosis Systolic blood pressure <120 mmHg Known allergy to glyceryl trinitrate (Transiderm-Nitro) patch Known sensitivity to Duoderm hydrocolloid dressing Planned for palliative care only Recent use of phosphodiesterase type 5 (PDE5) inhibitors, e.g., sildenafil (Viagra®) If a CT/MR scan has already been performed, then it shows a non-stroke lesion that explains the acute presentation Known previous enrolment in ENOS-2
Description of interventions	Active: Transdermal glyceryl trinitrate (GTN) 5 mg placed on back or shoulders and applied for 2 days Comparator: Transdermal Duoderm hydrocolloid dressing placed on back or shoulders and applied for 2 days.
Duration of study	The trial will last 36 months (until May 2024), with recruitment occurring over 31 months (until February 2024). Once enrolled, participants will be treated for 2 days, and followed-up at 90 (primary outcome).
Randomisation and blinding	Patients will be randomised (1:1) to receive either glyceryl trinitrate patches or sham patches. Randomisation will be performed by the Nottingham Stroke Trials Unit (STU) and involve computerised stratification by stroke type (IS or not known; ICH) and minimisation on age, severity, time, systolic blood pressure and candidate for or received reperfusion therapy. Patients, relatives, researchers and outcome assessors will be masked to treatment allocation.

Outcome measures	***Feasibility outcome*** • Recruitment of 100 IS patients, 20 ICH patients ***Mechanistic outcomes*** • Blood pressure (BP) and heart rate (HR) over first 2 days • Bloods ***Clinical outcomes*** *Day 2* • Impairment *Discharge or death* • Discharge destination *Day 90* • Functional outcome: modified Rankin Scale (mRS = primary clinical outcome) • Disability (Barthel index, BI), cognition (telephone TICS, MMSE), mood (Zung depression scale, ZDS), quality of life (Euro-QoL EQ-5D and EQ-VAS). **Safety outcomes** • Serious adverse events (SAEs) up to day 2 (i.e., 48 hours after 1st patch applied; fatal SAEs to day 90. • Death
Statistical methods	This is a feasibility trial, and the main analysis will be with descriptive statistics only. Counts will be summarised using N and %, and continuous variables will be summarised using means (standard deviation) or median [interquartile range] depending on their distribution. Mechanistic, clinical and safety outcomes will be compared between the treatment groups using binary logistic regression, Cox proportional hazards regression (death), ordinal logistic regression (mRS) or multiple linear regression (BI, TICS, t-MMSE, ZDS, EQ-5D, EQ-VAS, as appropriate, in exploratory analyses.

ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
A&E	Accident & Emergency
AI	Augmentation Index
ASPECTS	Alberta Stroke Program Early CT score
BI	Barthel Index
BP	Blood pressure
CE	Cardio-embolic
CF	Informed Consent Form
CI	Chief Investigator
CRF	Case Report Form
CT	Computerised Tomography
CTA	CT angiography
CTP	CT perfusion
DBP	Diastolic Blood Pressure
DMC	Data Monitoring Committee
eCRF	Electronic CRF
ED	Emergency Department
EMAS	East Midlands Ambulance Service
EQ-5D	Euro Quality of Life-5 Dimensions
EQ-VAS	Euro Quality of Life-Visual Analogue Scale
EOT	End of Trial
FAST	Face, Arm, Speech, Time test
GCP	Good Clinical Practice
GTN	Glyceryl Trinitrate
HR	Heart Rate
HUS	Health Utility Status
ICH	Intracerebral Haemorrhage
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IS	Ischaemic Stroke
ITT	Intention to treat
LACS	Lacunar syndrome
LAD	Large artery disease
MHRA	Medicines and Healthcare products Regulatory Agency
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance imaging
MRA	MR angiography
mRS	Modified Rankin Scale
NHS	National Health Service
NIHSS	National Institutes of Health Stroke Scale
NK	Not Known
NO	Nitric oxide
NTG	Nitroglycerin
NUH	Nottingham University Hospitals NHS Trust
PACS	Partial anterior circulation syndrome

PI	Principal Investigator (at a local centre)
PIS	Participant Information Sheet
POCS	Posterior circulation syndrome
PP	Per protocol
QP	Qualified Person
REC	Research Ethics Committee
R&D	Research and Development department
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SBP	Systolic Blood Pressure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
SVD	Small vessel disease
TACS	Total anterior circulation syndrome
TCD	Transcranial Doppler
TIA	Transient Ischaemic Attack
TICS	Telephone Interview Cognition Scale
TMC	Trial Management Committee
TSC	Trial Steering Committee
UoN	University of Nottingham
ZDS	Zung Depression Scale

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TRIAL / STUDY BACKGROUND INFORMATION AND RATIONALE

Stroke: Is common (life-time risk 1/5-6) and devastating (death 25%, dependency 40% at 1 year).[4] Acute treatment is limited to alteplase,[5] mechanical thrombectomy,[6] aspirin, hemicraniectomy and stroke unit care. Anticoagulation is ineffective,[7] neuroprotection unproven,[8,9] and there is no widely-agreed treatment for intracerebral haemorrhage (ICH) although lowering blood pressure (BP) may be beneficial.[10] Developing new interventions in hospitals has failed, in part, due to delayed treatment beyond the 'golden' hour after stroke. The management of physiological disequilibrium - BP,[11] oxygen, glucose,[12] cerebral oedema - remains unclear, and it is reasonable to hypothesise that their treatment, if warranted, should start rapidly after stroke onset.

High BP: Is common (80%) in patients with acute IS and ICH, and is associated independently with increased early recurrence and late death or dependency.[13-15] Whether lowering BP improves outcome through reducing expansion and recurrence, or worsens it through reducing cerebral blood flow (due to dysfunctional autoregulation) remains unclear, in part because most trials started treatment several hours after onset. When assessing functional outcome, trial results have varied from a strong positive trend (INTERACT-2: SBP 14 mmHg lower with intensive treatment in ICH [10]) through neutral effect (IMAGES: BP 4/3 mmHg lower with intravenous magnesium in mixed IS/ICH;[8] CATIS: systolic BP 9 mmHg lower with intensive treatment in IS;[16] ENCHANTED-BP SBP 5.5 mmHg lower with intensive BP-lowering in IS [17]) to strong negative trend (BEST: oral propranolol or atenolol in mixed IS/ICH;[18] INWEST: intravenous nimodipine in IS;[19,20] SCAST: BP 5/2 mmHg lower with oral candesartan in mixed IS/ICH [11]). Meta-analysis, and meta-regression of trial outcomes versus BP change, have not identified benefit.[21,22]

Nitric oxide (NO) and donors such as glyceryl trinitrate (GTN): Are candidate treatments for acute stroke and multiple mechanisms exist by which they might be effective; taken together, these actions may 'buy time' for the brain, protect it and prime patients for arterial reperfusion therapies:

- NO/GTN lowers BP in acute/subacute stroke [23] and so may 'move' patients down the epidemiological curve relating high BP and poor outcome.[13] This mechanism may be of particular relevance in ICH.
- NO dilates cerebral arteries (e.g. middle cerebral) so could increase 'front door' cerebral blood flow (CBF) and peri-lesional perfusion, as seen in the GTN-3 pilot trial.[24]
- NO dilates pial arteries (shown experimentally [25]) so might increase CBF via the 'back-door'.
- NO donors are neuroprotective in preclinical stroke,[26] especially if given early.
- Endogenous NO levels are low in acute stroke;[27] hence, administration will supplement low [NO].
- GTN may 'prime' patients for rt-PA by lowering their BP so that more can be treated, and more rapidly after hospital arrival. RIGHT showed non-significant trends for these.[28]
- GTN, through cerebral vasodilation, may increase access of alteplase to obstructing clot and therefore increase the effectiveness of thrombolysis, i.e., GTN might be additive to rt-PA.

Several NO donors are licensed in the UK and are used widely in patients with ischaemic heart disease, heart failure and severe hypertension; these include intravenous sodium nitroprusside and transdermal glyceryl trinitrate. One uncontrolled pilot study found that sodium nitroprusside lowered BP without altering cerebral perfusion (assessed using CT SPECT), and attenuated platelet function, in patients with recent ischaemic stroke.[29] Four

phase II and two phase III randomised controlled trials have assessed transdermal glyceryl trinitrate in patients with acute stroke (IS and ICH) (Table 1).

Table 1. Characteristics of completed trials of transdermal glyceryl trinitrate.

	GTN-1 [30]	GTN-2 [31]	GTN-3 [24]	RIGHT [28,32,33]	ENOS [2,34-36]	RIGHT-2 [3,37-40]
Setting	Hospital	Hospital	Hospital	Pre-hospital	Hospital	Pre-hospital
Time window (hr)	<120 hours	<72 hours	<120 hours	<4 hours	<48 hours	<4 hours
Stroke type	IS/ICH	IS/ICH	IS/ICH	IS/ICH	IS/ICH	IS/ICH
SBP range (mmHg)	No limits	100-230	140-220	>140	140-220	>120
Treatment blinding	Double-blind	Open-label	Single-blind	Single-blind	Single-blind	Single-blind
GTN dose (mg)	5	5/10	5	5	5	5
Thrombolysis given	N/A	N/A	N/A	After GTN	Before GTN	After GTN
Sample size						
Intended	38	90	18	80	>3500	850
Achieved	37	90	18	41	4011	1149

ICH: intracerebral haemorrhage; IS: ischaemic stroke; N/A: not applicable; SBP: systolic blood pressure

GTN lowered BP, increased heart rate, and did not alter cerebral blood flow (assessed using xenon CT) or platelet function.[24,28,30-33] The safety and efficacy of GTN in patients with acute stroke (IS, ICH) has been assessed in two large phase III trials.

International hospital-based MRC ENOS trial:[2] In comparison with no GTN, transdermal GTN was not associated with any difference in functional outcome (mRS), disability/Activities of Daily Living (BI), cognition (tMMSE, TICS, animal naming), mood (ZDS) or quality of life (EQ-5D/HUS, EQ-VAS) (Table 2). No safety issues were found with GTN.[2]

In the subgroup of patients randomised within 6 hours of ictus (average time from event to randomisation 263 minutes) (identified here as 'ENOS-early'), treatment with GTN was associated with reduced death, and improved functional outcome (mRS, BI) and quality of life (Table 2).[1]

UK ambulance-based BHF RIGHT-2 trial: The average time to treatment was 70 minutes. In comparison with sham, transdermal GTN was not associated with any differences in functional outcome (mRS), disability/Activities of Daily Living (BI), cognition (tMMSE, TICS, animal naming), mood (ZDS) or quality of life (EQ-5D/HUS, EQ-VAS).

Table 2. Outcomes in patients treated with GTN versus no GTN/sham
Data are number (%), median [interquartile range] or mean (standard deviation). Analysis with binary logistic regression (odds ratio), ordinal logistic regression (odds ratio) or multiple regression (mean difference).

Outcome measure	GTN	No GTN	OR/MD (95% CI)	p
ENOS (OTR 0-48 hours) [2]				
Modified Rankin Scale (/6)	3 [3]	3 [3]	1.01 (0.91, 1.13)	0.83
Barthel Index (/100)	66 (38)	63 (39)	2.18 (-0.23, 4.59)	0.11
EQ-VAS (/100)	57 (31)	56 (32)	0.8 (-1.3, 2.9)	0.70
Death (%)	233 (12)	263 (13)	0.89 (0.72, 1.10)	0.27
ENOS-early (OTR 0-6 hours) [1]				
Modified Rankin Scale (/6)	3 [3]	3 [3]	**0.53 (0.34, 0.82)**	**<0.001**
Barthel Index (/100)	74 (34)	60 (41)	**14 (4.6, 22.5)**	**0.01**
EQ-VAS (/100)	62 (29)	53 (35)	**9.6 (1.8, 17.5)**	**0.03**
Death (%)	11 (8)	26 (20)	**0.35 (0.13, 0.96)**	**0.04**
RIGHT-2 (OTR 0-4 hours) [3]				
Modified Rankin Scale (/6)	3 [3]	3 [3]	1.25 (0.97, 1.60)	0.083
Barthel Index (/100)	56 (45)	58 (44)	-3 (-8, 2)	0.29
EQ-VAS (/100)	45 (34)	47 (32)	-0.9 (-5.1, 3.2)	0.66
Death (%)	97 (23)	79 (19)	1.24 (0.91, 1. 86)	0.17

CI: confidence intervals; EQ-VAS: EuroQoL-Visual Analogue Scale; ICH: intracerebral haemorrhage; IS: ischaemic stroke; MD: mean difference; N/A: not applicable; OR: odds ratio; SBP: systolic blood pressure.

The following summary observations can be made based on data from the four phase II and two phase III GTN trials:

- Transdermal administration is advantageous since oral treatment is confounded by dysphagia in 50% of patients with acute stroke, whilst intravenous therapy requires intensive monitoring. Additionally, treatment can be stopped and restarted according to need.
- Peak concentrations of GTN are achieved by 1-2 hours.[41]
- Transdermal GTN lowers systolic BP significantly by 15, 60 and 120 minutes.[24,28]
- Transdermal GTN lowers central and peripheral SBP, DBP and pulse pressure; peak systolic BP and augmentation index; and 24-hour BP in both dipping and non-dipping patients.[24,28,31,42,43]
- Transdermal GTN is feasible to administer, well tolerated, and safe when given early after acute stroke.[2,3,24,28,31,42,44]
- GTN does not alter platelet function; hence, it can be given in ICH as well as IS.[42]
- GTN does not reduce cerebral blood flow.[24,31]
- GTN is safe in patients with severe carotid stenosis.[45]

Further, the effect of GTN on functional outcome appears to be time-dependent when administered after stroke:

- <2.0 hours: No effect; indeed, GTN may cause harm in ICH in this ultra-acute period (interpretation from RIGHT-2 **[3,40]**)
- 2.5 to 5.5 hours: May improve functional outcome (interpretation from ENOS-early [1] and meta-analysis, Figures 1 , 2).
- >6 hours: No effect (interpretation from GTN 1/2/3 and ENOS and an individual patient data meta-analysis [46]).

(The positive effect of GTN on functional outcome in the phase II RIGHT trial is ignored here because it was small.)

Figure 1. Meta-analysis of GTN versus no GTN on end of trial functional outcome in patients treated within 6 hours of stroke onset (from [3])

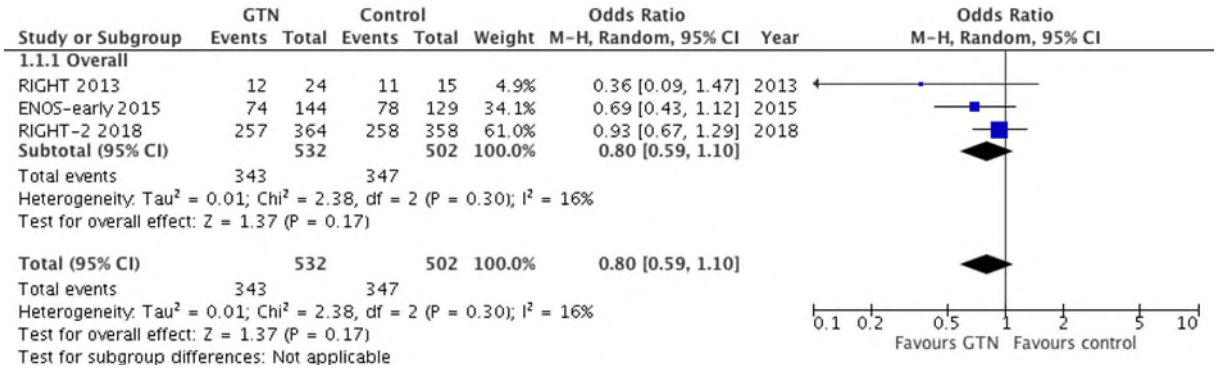
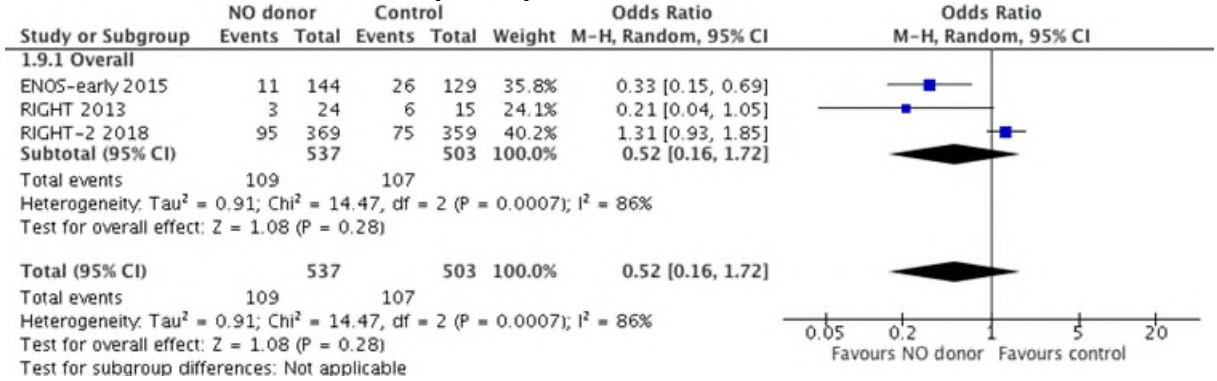


Figure 2. Meta-analysis of GTN versus no GTN on end of trial death in patients treated within 6 hours of stroke onset (from [3])



In summary, GTN has been associated with beneficial effects on function and other clinical outcomes in hospitalised patients treated up to 6 hours after ictus. However, benefit was not seen in patients treated prior to hospital admission and there was weak evidence that ultra-acute treatment within 2 hours was associated within harm. As a result, ENOS-2 will assess, in a novel design, the feasibility of recruiting patients between 3 and 5 hours after ictus; if feasible, a larger safety and efficacy trial will be performed in this group of patients.

ENOS-2 will assess the feasibility, safety, and efficacy of hospital-delivered GTN when administered hyper-acutely after stroke. Five of the six GTN trials had a lower limit for systolic blood pressure of ≥100 mmHg,[31] >120 mmHg [3] or ≥140 mmHg;[2,24,28] the first study had no lower limit.[42] Since several potential mechanisms by which GTN might work are BP-independent, ENOS-2 will follow the RIGHT-2 protocol and include patients with high-normal BP as well as high BP, with a lower limit set to ≥ 120 mmHg. This has the advantage that the results will apply to a wider population of stroke patients and include more patients with severe stroke (some of whom have SBP <140 mmHg).

DETAILS OF INVESTIGATIONAL MEDICINAL PRODUCT(S)

Description

The investigational medicinal product (IMP) is transdermal glyceryl trinitrate patch (GTN, - Transiderm-Nitro '5' (Novartis). GTN is also known as nitroglycerin (NTG). One patch will be given on 2 consecutive days. Further chemical and pharmacological properties of the GTN patch - Transiderm-Nitro '5' are provided in a separate Summary of Product Characteristics document (SmPC).

The GTN patch will be placed on the shoulders or back and the position rotated daily. GTN patches will be covered with a gauze dressing to mask the patient to treatment.[24,32,34]

Manufacture

The GTN patch Marketing Authorisation Holder is Novartis Pharmaceuticals Ltd.
PL 00101/0464

Packaging and labelling

Active patches will not be removed from their primary packaging. For blinding purposes, active patches will be packed with a gauze dressing into a plain opaque pouch which will be heat sealed by Pharmacy Production, Nottingham University Hospitals NHS Trust MIA(IMP): 19162. The manufacture will be in accordance with cGMP. Groups of 2 pouches will be assigned a pack number and these pouches will be packed into a labelled carton with the same pack number. The outer pouches and carton will be labelled according to Annex 13 - the Rules Governing Medicinal Products in the European Union, Volume 4. Labels will state usage instructions and storage conditions for the IMP.

The final product will be QP released by the designated person to provide blinded trial treatment packs for use in the trial.

Storage, dispensing and return

NUH Clinical Trial Pharmacy will act as the central distribution pharmacy and receive, and store blinded packs from Pharmacy Production. Pharmacy will send packs to the participating stroke units.

Each participating research coordinator will take and sign-out one numbered pack following consent and randomisation. When a pack is assigned to a participant, the coordinator will add the Participant's name and date dispensed to the label. If the pack has been assigned, the coordinator will complete the Use/Non-use Form. If the pack has been assigned but the IMP was not used (e.g., if the participant changed their mind after consenting but before application of the patch), the coordinator will explain on the Use/Non-use Form why treatment was not administered and return the pack to the Nottingham Pharmacy.

Trial treatment must not be used for any other purpose than the present study. Any part-used packs will be returned to NUH for disposal. Returned trial medication that has been assigned to a participant must not be re-dispensed to a different participant.

Known Side Effects

Table 3. Known side effects of the IMP

Nervous System Disorders: Common: Very rare:	 Headache Dizziness
Cardiac Disorders: Rare:	 Tachycardia
Vascular Disorders: Rare:	 Orthostatic hypotension, flushing
Gastrointestinal Disorders: Very Common:	 Nausea, vomiting
Skin and subcutaneous tissue disorders: Uncommon:	 Contact dermatitis
General disorders and administration site conditions: Uncommon:	 Application site erythema, pruritus, burning, irritation.
Investigations: Rare:	 Heart rate increase

NB. Very common (≥ 1/10); common (≥ 1/100, <1/10); uncommon (≥1/1000, <1/100); rare (≥ 1/10,000, <1/1000); very rare (<1/10,000), including isolated reports.

Contraindications to the IMP are as follows:

- Known hypersensitivity to glyceryl trinitrate/nitroglycerin, and related organic nitrates or any excipient.
- Acute circulatory failure associated with marked hypotension (shock).
- Conditions associated with elevated intracranial pressure.
- Myocardial insufficiency due to obstruction, as in aortic or mitral stenosis, or constrictive pericarditis.
- Concomitant use with a phosphodiesterase type 5 (PDE5) inhibitor such as sildenafil (Viagra®) because PDE5 inhibitors may amplify the vasodilatory effects of GTN resulting in severe hypotension.
- Severe hypotension (systolic blood pressure less than 90 mmHg).
- Severe hypovolaemia.

The following interactions are possible:

- Concomitant administration with other vasodilators e.g., PDE5 inhibitors such as sildenafil potentiate the blood pressure lowering effects of GTN.
- Concomitant treatment with calcium antagonists, ACE inhibitors, beta-blockers, diuretics, antihypertensives, tricyclic antidepressants and major tranquillisers may potentiate the blood pressure-lowering effect of GTN, as may alcohol.
- Concurrent administration with dihydroergotamine may increase the bioavailability of dihydroergotamine. This warrants special attention in patients with coronary artery disease, because dihydroergotamine antagonises the effect of GTN/nitroglycerin and may lead to coronary vasoconstriction.
- Non-steroidal anti-inflammatory drugs, except acetyl salicylic acid, may diminish the therapeutic response of Transiderm-Nitro.

- Concurrent administration with amifostine and acetyl salicylic acid may potentiate the blood pressure lowering effects of Transiderm-Nitro.

Reference source: SPC:
http://www.medicines.org.uk/emc/medicine/1333/SPC/Transiderm-Nitro 5 and 10

Placebo

There is no matching placebo. The control group will not receive GTN, and matching placebo GTN patches are not available. Instead, a sham patch of similar size to the GTN patch (Duoderm – a hydrocolloid dressing of size 4.4cm x 3.8cm) will be used, Like the GTN patch, it will be placed on the shoulders or back and the position rotated daily. Duoderm patches are manufactured by Convatec and are presented in individually wrapped packages. They do not have identifying information on them. Sham-Duoderm patches will be covered by a gauze dressing to conceal treatment allocation.[24,32,34]

Trial packaging

The placebo packs will be manufactured, supplied, packaged, labelled and QP released to provide blinded treatment packs and distributed in the same way as the active IMP. Storage, dispensing and return of the sham patch will be the same as the active IMP.

Known side effects of placebo patches

There is the possibility of allergic reactions to the product or its components. Patients who have a known sensitivity or allergy to the product or its components should therefore not participate in the study.

Reference Safety Information: Section 4.8 Transiderm Nitro SmPC

TRIAL / STUDY OBJECTIVES AND PURPOSE

PURPOSE

- The purpose of the study is to determine whether it is feasible to recruit patients and administer GTN within the 3-5-hour time window after the onset of stroke.

PRIMARY OBJECTIVE

- To assess the feasibility of recruiting, randomising, and treating patients with GTN vs sham to inform a definitive trial.

SECONDARY OBJECTIVES

- To determine signals of efficacy on whether GTN reduces disability, low mood, poor cognition, and low quality of life.
- To investigate whether there is a difference between the two groups in blood pressure measured over the two days of monitoring.

- To investigate whether specific genetic characteristics are associated with outcome. Potential genetic markers include nitric oxide synthase polymorphisms, but others will be studied as relevant in searches of the scientific literature.
- To investigate whether there is a difference in blood biomarkers between the two groups and whether biomarkers may be associated with outcome. Potential biomarkers include S-100 / nitric oxide (NOx) but others will be studied as relevant in searches of the scientific literature.

TRIAL / STUDY DESIGN

TRIAL CONFIGURATION

Prospective parallel-group randomised sham-controlled phase II feasibility and safety trial of GTN versus sham given initially between 3 and 5 hours after stroke, and then at 09.00 on the next day.

Primary endpoint

The primary end point of the study is the feasibility of recruiting and treating 120 patients (100 IS, 20 ICH) between 3 and 5 hours after stroke.

Secondary endpoints

Hospital admission:
- Neurological impairment (NIHSS)
- Systolic and diastolic blood pressure, heart rate.
- Proportion of participants with systolic blood pressure <185 mmHg.
- Feeding and dysphagia (dysphagia severity rating scale)
- Stroke lesion size on brain scan (non-contrast CT or T2 MR).
- Amount of cerebral arterial patency on brain scan (CT or MR angiography).

Hospital utilisation:
- Open-label blood pressure lowering.
- Intravenous thrombolysis.
- Mechanical thrombectomy.
- Hyperacute stroke unit.
- Stroke Rehabilitation Unit.
- Physiotherapy.
- Occupational therapy.
- Speech & language therapy.
- Surgery for IS - Hemicraniectomy.
- Surgery for ICH.
- Days in intensive/critical care unit.

At day 2:
- Systolic and diastolic blood pressure, heart rate.

At day 2: Biomarkers
- Blood biomarkers (exact measures to be determined by literature review prior to measurement but examples include S-100, NOx).
- Genetic markers (exact measures to be determined by literature review prior to measurement but examples include NO synthase polymorphisms).

At day 2 (or discharge if sooner):
- Neurological impairment (NIHSS).
- Stroke recurrence.
- Neurological deterioration from baseline (NIHSS ≥4 points, or ≥2-point increase in any domain).
- Feeding and dysphagia (DSRS).

At discharge/death
- Length of stay in hospital
- Patient disposition.

At day 90 by telephone (or post):
- Dependency – modified Rankin Scale (primary endpoint at Day 90).
- Disability/Activities of Daily Living - Barthel Index (BI).
- Quality of life - Health Utility Status (HUS, derived from EuroQoL-5D), EQ-Visual Analogue Scale (EQ-VAS).
- Cognition - telephone-MMSE, Telephone Interview Cognition Scale (TICS), animal naming.
- Mood - Zung Depression Scale.
- Patient disposition (died, institution/in hospital, home).

Safety endpoints

By day 2
- Any serious adverse event.
- Headache.
- Infection (pneumonia/chest, urinary tract, other).
- Hypotension requiring clinical intervention.
- Hypertension requiring clinical intervention.

From day 3 to day 90
- Any fatal serious adverse event.

Data on stroke recurrence and acute coronary syndrome, termed safety outcome events, will be collected up to day 90.

Stopping rules and discontinuation

The DMC review unblind data twice yearly in respect of safety and efficacy and consider the study in the context of other trials of altering BP in stroke. Since the main aim of the study is to assess feasibility, the trial will not be discontinued unless the safety of participants is likely to be compromised. The DMC also monitor SAEs and neurological deterioration, and outcome in IS and ICH separately.

RANDOMISATION AND BLINDING

All participants eligible for inclusion will be randomised centrally using a secure internet site in real-time. Randomisation will be 1:1 GTN: sham and involve computerised stratification by stroke type (ischaemic stroke or not known vs ICH) and minimisation on age, severity, time, systolic blood pressure and candidate for or received reperfusion therapy (alteplase, and/or mechanical thrombectomy. This approach ensures concealment of allocation, minimises differences in key baseline prognostic variables, and slightly improves statistical power (16). Patients, relatives, researchers, and outcome assessors will be masked to treatment allocation.

In order to minimise selection bias, a participant must be enrolled in the study before they are randomised on the internet site. Randomisation will allocate a number corresponding to a treatment pack and the participant will receive treatment from the allocated numbered pack.

In the event of computer failure (for example: server failure), investigators will follow the working practice document for computer system disaster recovery, which will allow the participant to be randomised following standardised operating procedure.

Personnel applying the GTN/sham patches will not be blinded to the treatment allocation as the sachets around the patches are different and carry the manufacturers identifying information.

Maintenance of randomisation codes and procedures for breaking code

Although both the GTN and sham patches have no identifying information on them, they look slightly different, and it will be obvious to nurses when administering treatment. Since GTN and sham patches will be placed on the participant's shoulder or back, it will be difficult for them to recognise the treatment that they have received.

In order to minimise bias that could be introduced through knowledge of what the participant has received, unmasked staff will be kept to a minimum and be asked not to reveal the treatment to anyone. In addition, the primary end-point of the study will be recorded by personnel masked to treatment assignment.

Unblinding

In the event of breaking the treatment code this will normally be recorded as part of managing a SAE (see below for more details) and such actions will be reported in a timely manner. The investigator may unblind themselves by removing the gauze from the patch. The GTN is identifiable by medical staff. The Chief Investigator (delegated the sponsor's responsibilities) shall be informed immediately (within 24 hours) of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

TRIAL MANAGEMENT

Day-to-day management of the trial will be the responsibility of the Trial Management Committee (TMC). The TMC will report to the Trial Steering Committee (TSC). An independent Data Monitoring Committee (DMC) will monitor the safety of participants and will advise the TSC on safety. Trial co-ordination will pass through the Nottingham Stroke Trials Unit and members of the TMC.

The Chief Investigator has overall responsibility for the study and shall oversee all study management.

The data custodian will be the Chief Investigator.

Trial Management Committee (TMC)

The Trial Management Committee will include the Trial Manager (who will chair meetings), Chief Investigator, Trial Coordinators, Trial Statistician, Trial Programmer, and other project staff. This group, based at the Nottingham Stroke Trials Unit, will meet at least monthly or as necessary.

Trial Steering Committee (TSC)

The Trial Steering Committee will provide oversight of the trial. It will meet (in person or by telephone conference) prior to commencement of the trial, and then at regular intervals (at least annually) until completion. The TSC will be chaired by an independent member and comprise two other independent members, the Grant Applicants (including the lay member), and representatives of the sponsor and funder. The standard University of Nottingham Trial Steering Committee Charter and Contracts will be used.

Specific tasks of the TSC are:

- To approve the trial protocol.
- To approve necessary changes to the protocol based on considerations of feasibility and practicability.
- To receive and review reports from the DMC.
- To resolve problems brought to it by the co-ordinating centre and TMC.
- To lead on publication of the trial results.

Data Monitoring Committee (DMC)

An independent Data Monitoring Committee will be established. The DMC will receive safety reports every six months, or more frequently if requested, and perform unmasked reviews of efficacy and safety data. No interim analysis will be performed.

The standard University of Nottingham Data Monitoring Committee Charter and Contracts will be used containing details of membership, terms and conditions and full details of stopping guidelines. The DMC will report their assessment to the independent chair of the TSC and the CI. They will not, however, provide specific unmasked results to the TSC or Chief Investigator.

DURATION OF THE TRIAL / STUDY AND PARTICIPANT INVOLVEMENT

Participant Duration: The study will recruit patients for 31 months. The participant's involvement in the trial will last for 3 months, from randomisation (day 1) until final follow-up at 90 days. Treatment will last 2 days (enrolment day and next day).

Study Duration: The study will recruit patients for 31 months. The participant's involvement in the trial will last for 3 months, from randomisation (day 1) until final follow-up at 90 days.

Treatment will last 2 days (enrolment day and next day).Total study duration will be 36 months.
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End of the Trial

The end of the study will be the last treatment period and follow up (Day 90) of the last participant (LPLV).

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

The trial setting is in secondary care at NIHR Clinical Research Network sites with dedicated Research Coordinators who will facilitate recruitment and follow-up to discharge or death. Adoption of the trial from the NIHR Clinical Research Network has been sought.

Participants will be recruited from the hyperacute stroke unit or emergency department. The initial approach will be from a member of the patient's usual care team, which may include Investigators and Research Coordinators.

The investigator or their nominee, e.g., from the research team or a member of the participant's usual care team, will inform the participant or their nominated representative (other individual or other body with appropriate jurisdiction), of all aspects pertaining to participation in the study.

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the trial, the participant information sheets, and consent forms, but the consent forms and information sheets will not be available printed in other languages.

In view of the narrow time window for enrolment and treatment (2 hours), to ensure thrombolysis is not delayed, and the known safety of the intervention beyond 2 hours after stroke onset; verbal consent will be taken from the participants themselves if they have capacity, or proxy consent from a relative/friend where the participant does not have capacity. In participants who lack capacity and have no relatives present a patch may need to be applied in order to prevent delay in performing CT scan /thrombolysis. The trial will use emergency waiver of consent in accordance with statutory instrument 2006, No. 2984, regulation 2, where participants lack capacity to consent for themselves, all verbal consent will be documented in the medical notes.

Assessment of capacity

The investigator will tell the patient that:
•they have had a suspected stroke
•they have higher than ideal blood pressure that needs to be lowered
•they can be in a trial where they will have patches placed on their back for 2 days which will either contain medicine that will lower their blood pressure, or will not contain the medicine

They will then ask the patient 3 questions to assess their capacity (diagnosis stroke, problem–blood pressure needs lowering, treatment patch). If the patient is able to answer the three questions correctly, capacity is assumed
1. If patient has capacity, they may give verbal consent.
2. If the patient does not have capacity, the investigator will explain the trial to a relative/friend if they are immediately available. The relative/friend who is able to represent patients' views and wishes may then give verbal proxy consent, which will be documented in

the participants medical notes.

Written consent will be obtained after treatment for hospital and community-based follow-up. If the participant has capacity, they will be asked to read and sign the short pictorial combined information sheet and consent form; if they lack capacity a relative/friend will be asked to read and sign the short pictorial combined relative information sheet and consent form.

It will be explained to the potential participant that entry into the trial is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal, it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Eligibility criteria

Inclusion criteria

- Adults (≥18 years)
- Presentation compatible with hyperacute stroke syndrome
- One or more of the following symptoms present at time of enrolment: Dysphasia, neglect (NIHSS 1-2), hemianopia (NIHSS 1-3), or limb weakness (NIHSS on affected arm and/or leg 1-4)
- Treatment can be commenced between 3 and 5 hours from onset of symptoms (for patients with wake-up stroke, treatment no more than 5 hours after patient awakens)
- Systolic BP ≥120 mmHg
- If a CT/MR scan has already been performed, then it shows acute intracerebral haemorrhage or ischaemic stroke, or is normal. (If a CT scan has not been performed then it should be performed as soon as possible after treatment)
- For participants who lack capacity to consent for themselves and have no relative/friend available: Waiver of consent for treatment to ensure GTN given in 3-5-hour time-window (and thrombolysis not delayed if ischaemic stroke)

Exclusion criteria

- mRS ≥4
- Hypotension or shock (systolic <120 mmHg)
- BP Glucose (BM stix or equivalent) <3 mmol/l
- Glasgow coma scale ≤8
- Witnessed seizure at presentation
- Known life expectancy <6 months
- Patient presenting with sensory symptoms only
- Known stroke mimic, aneurysmal subarachnoid haemorrhage, or haemorrhage due to venous thrombosis
- Known allergy to glyceryl trinitrate (Transiderm-Nitro) patch
- Known sensitivity to Duoderm hydrocolloid dressing
- Planned for palliative care only
- Recent use of phosphodiesterase type 5 (PDE5) inhibitors, e.g., sildenafil (Viagra®)
- If a CT/MR scan has already been performed, then it shows a non-stroke lesion that explains the acute presentation
- Known previous enrolment in ENOS-2

Expected duration of participant participation

Study participants will be participating in the study for 90 days.

Removal of participants from therapy or assessments

Participation in the trial is voluntary and patients are free to stop treatment, miss follow-up visits or withdraw completely from the trial at any stage without giving a reason.

- ***Study medication***: This may be stopped at any time by the investigators or any treating clinician if deemed in the patient's best interest. Treatment (with GTN/sham) will be given on top of 'best medical care'. Stopping study medication does not constitute withdrawal and follow-ups should be continued as per this protocol.
- ***Follow-ups***: These may be missed but this does not constitute withdrawal from the trial and the remaining follow-up visits should be continued as per this protocol.
- ***Withdrawal from trial***: Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Informed consent

Stroke, including ischaemic stroke and intracerebral haemorrhage, is a medical emergency with limited treatment options. Further, rapid deterioration can occur early, leading to brain damage, long term disability or death. Stroke can cause significant brain injury and many patients may not be physically or mentally capable of giving informed consent to participate in a clinical trial. There is evidence from previous trials in stroke (involving >4000 patients in ENOS and three hospital pilot trials) that transdermal GTN (5 mg) is safe if given after 2.5 hours. The need for urgent treatment, in an attempt to improve outcome and reduce deterioration, means that it would be inappropriate to delay treatment until fully informed consent can be obtained from an incapacitated patient. Further, patients with IS should be considered for intravenous thrombolysis and this must take precedence over any trial that requires consent for treatment.

Patient able to provide consent

All participants who have capacity will provide verbal consent followed by written informed consent. The Consent Form will be signed and dated by the participant after treatment and before follow-up. The Investigator (or nominee) will explain the details of the trial and provide a Pictorial Information Sheet. The Pictorial Information Sheet has been specifically designed in order to speed up the process in this emergency condition. This approach has been successfully used in TICH-2 (Ref: ISRCTN93732214) and RIGHT-2 study (Ref: ISRCTN26986053) and is supported by stroke survivors (including those in the Nottingham Stroke Research Partnership Group). The Investigator will answer any questions that the participant has concerning study participation and follow-up. If requested, a more detailed information sheet will be provided. Participants will be given as long as they need to consider whether to consent to follow up.

If the participant is unable to write (e.g., in the presence of dominant hand weakness, ataxia or dyspraxia), witnessed verbal consent (or any mark made by the participant as intent to sign) may be recorded on the consent form by someone unconnected with the study.

Participants may discontinue treatment either at their own request or if it is felt in their best interest by the attending physician (for whatever reason); these participants remain in the trial and should have follow-up performed as per protocol. Participants may decline to have a follow up visit but should remain in the trial unless they specifically ask to be withdrawn. Participants may withdraw from the trial either at their own request (if they regain capacity) or at the request Investigator. Participants who withdraw from the trial will be informed that data already collected prior to withdrawal cannot be deleted and may still be used in final analyses

Consent for treatment where participants lack capacity

Participants who lack capacity but have a relative/friend present will be asked to provide verbal consent followed by written consent or proxy written consent after treatment for hospital and community-based follow-up. Ability to give consent will be determined by the participant's attending stroke physician or investigator as previously described:

- ***Proxy consent***: Participants who regain capacity will be approached to give consent for further follow up, or to withdraw from the trial. Participants who die after treatment and before consent will have their data used without proxy consent since this avoids unnecessary stress to grieving relatives.

Patient unable to give consent for follow-up (after treatment and before going home?)
If the potential participant is unable to give meaningful consent (e.g., in cases of dysphasia, confusion, or reduced conscious level), a relative or close friend able to represent the patient's views and wishes will be approached with the pictorial information about the trial; this approach was used in RIGHT-2 (Ref: ISRCTN26986053). If requested, a more detailed information sheet will be provided. The Consent Form will be signed and dated by the legal personal nominee before the patient enters the trial. If the relative objects to the inclusion of the patient in the trial, their views will be respected, and the patient will not be enrolled.

If relatives are not physically present but are available and happy to speak on the telephone, the same procedure will be followed with the exception that the paper consent form will be countersigned by a witness unconnected with the study and signed by the relative upon their arrival to the hospital site. If the relative is unhappy to speak on the telephone or unable to decide, the patient will not be followed-up.

If the participant recovers capacity, their decision to continue or withdraw will overrule the decision of the nominee.

Other information on consent
The requirements of the relevant ethics committee will be adhered to at all times.
Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended consent form, which will be signed by the participant.

Where the patient is being assessed and treated via telemedicine (as is often standard care in many stroke services out of hours) by a member of the medical team who is appropriately trained and listed on the delegation log, the process is as above, with the exception that the paper consent form will be countersigned by a witness unconnected with the study and signed by the investigator upon their return to the hospital site. If the patient does not wish to decide via telemedicine they will not be enrolled. This process has been successfully used in TICH-2 study (Ref: ISRCTN93732214).

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the trial, the participant information sheets, and consent forms, for follow up consent. Due to the rapid consent process, interpreters will not be used for verbal consent. The consent forms and information sheets will not be available in other languages.

It will be explained to the potential participant or their relative/friend that entry into the trial is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal, it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

TRIAL / STUDY TREATMENT AND REGIMEN

Trial treatment is administered as transdermal glyceryl trinitrate 5 mg patches. Sham treatment comprises a Duoderm dressing. Both are covered with a gauze dressing

Participants will be assessed in hospital (see flow chart)
- Before treatment on day 0.
- 1 hour after treatment on day 0.
- 1 hour after treatment on day 1.
- At discharge/death.

Follow up at day 90 will be via a telephone interview (as in ENOS, RIGHT-2). Researchers will not contact the participant or their family directly at day 90; they will first contact the participant's general practitioner (GP) or obtain information through the Medical Research Information Service/NHS Information Centre to check their health status. Permission to contact the GP at day 90 will be sought at the time of consent. In the event that a participant has been unable to be contacted to perform their follow-up at day 90, researchers will send a letter to their GP to request that they (or another healthcare professional if more appropriate), confirm an appropriate mRS value for the participant and the date on which this value was determined. This aims to reduce data loss associated with participants being lost to follow up.

Brain imaging (CT head scan) will be performed at baseline (Day 0):
- As part of routine clinical management prior to or soon after enrolment – all acute stroke patients have a CT head scan performed on admission to hospital for diagnostic purposes, this including a non-contrast CT scan +/- CT angiogram.

Participant measures

Assessments	**Screen**	**Day 0 Baseline Pre-treatment**	**Day 0 Post-treatment**	**Day 1: During treatment**	**Day 2: after treatment**	**Dis-charge, death**	**Day 90: Tele-phone**
Face-to-face							
Clinical assessment	X[1]				**X**	X	
Eligibility screen	**X**						

CT Scan		X[1]	or X[1]				
Consent		X		or X	or X		
Randomisation		X					
GTN/Sham patch			X	X			
mRS		X[2]					
U&E FBC		X[1]					
Blood pressure		X[1]	X	X			
NIHSS		X[1]			X		
DSRS		X			X		
Blood Biomarkers		X[3]			X[3]		
Hospital utilisation						X[4]	
Listed events				X[5]	X[5]		
SAEs, non-fatal			X	X	X		
SAEs, fatal				X	X	X	X
mRS							X
Barthel Index							X
EuroQoL (EQ5D)							X
Cognition (t-MMSE, TICS)							X
Mood (ZDS)							X

CT: Computerised tomography
NIHSS: National Institutes of Health stroke scale
MMSE: mini mental state examination
mRS modified Rankin scale
SAEs: serious adverse events

1. Routine as part of clinical practice
2. Pre-morbid mRS
3. Biomarkers: soluble markers, genetics
4. Open-label blood pressure lowering, intravenous thrombolysis, mechanical thrombectomy, hyperacute stroke unit, Stroke Rehabilitation Unit, physiotherapy, occupational therapy, speech & language therapy, surgery for IS – hemicraniectomy, surgery for ICH, days in intensive/critical care unit.
5. Hypotension, hypertension, headache, infection
6. mRS, BI, EQ-5D, EQ-VAS, t-MMSE, TICS and ZDS scales are given in Appendices A-H.
7. Neuroimaging scales are listed in Appendices I and J.

Concomitant and Rescue Medications and Treatments

The intervention (GTN or sham) will be given in addition to routine care. There are no prohibited concomitant treatments.

No publications have been found that would suggest a contraindication as a result of the interaction between the IMP and the COVID-19 vaccine.

Compliance

Compliance will be assessed by examining the participant's drug chart and recording evidence of treatment administration. Adherence will be recorded on the case report forms after treatment has been completed (day 2).

Accountability for drugs & placebos

The pharmacist will maintain records of the dispensing of the IMP from pharmacy to stroke unit/ED and the clinical and research team will record administration of the IMP to the patient. Dispensing details will be recorded on each participant's CRF. Unused and partially used supplies will be returned to pharmacy. This will be recorded in the pharmacy study log.

Management of study drug overdose

No specific antidotes are available. The study drug is administered as a transdermal patch by qualified nursing/medical staff so the potential for overdose is not anticipated.

Urgent Safety Measures

Any urgent safety measure relating to a Clinical Trial of an Investigational Medicinal Product (CTIMP) should be communicated to the MHRA immediately. They advise that sponsors phone the MHRA Clinical Trial Unit and discuss the event with a safety scientist. The sponsor must then follow-up with notification in writing within three days of the action being taken. The notification should be in the form of a substantial amendment and should describe the event, the measures taken and justification for the measures taken. Please see the section on adverse event reporting.

Protocol Deviations and Violations

The study should be conducted in accordance with the approved protocol and changes to the protocol will only be made to protect the safety, rights, or welfare of the subject.

Protocol Deviation
A Protocol Deviation is a deviation from the protocol that affects the conduct of the trial in a usually minor way. This includes any deviation from the trial protocol that is not listed as a Protocol Violation.

Protocol Violation
A protocol violation is a usually major deviation from the expected trial regimen such as where a participant is enrolled in spite of not fulfilling all the inclusion and exclusion criteria, or where deviations from the protocol could affect the trial delivery or interpretation significantly.

All protocol deviations and violations must be reported immediately to the Chief Investigator, via the online electronic case report form. The CI will notify the Sponsor if a deviation or violation has an impact on participant safety or integrity of the trial data. The Sponsor will advise on appropriate measures to address the occurrence, which may include reporting of a serious GCP breach, internal audit of the trial and seeking counsel of the trial committees.

Examples of protocol violations are given below but this list is not exhaustive:

- Day 2 follow-up performed >7 days past due date

- Death/discharge form entered >14 days after event
- Day 90 follow-up performed <80 days or >120 days past due date
- Participant enrolled in spite of not fulfilling all the inclusion and exclusion criteria
- Aneurysmal subarachnoid haemorrhage known at time of randomisation
- Non-stroke diagnosis, known at time of randomisation
- Failure to complete SAEs where appropriate
- Management of IMP – treatment pack lost

Criteria for terminating trial

The trial may be terminated by either the TSC, the sponsor or the funder if there is overwhelming evidence of major safety concerns, new information becomes available that makes the trial unsafe or irrelevant, or there are issues with trial conduct (e.g., poor recruitment, loss of resources).

RADIATION EXPOSURE

Details of diagnostic or therapeutic ionising radiation

Participants will have 1 or more CT head scans:

- ***Clinical***: A routine clinical CT head scan comprising non-contrast CT shortly after presentation to hospital according to the local clinical imaging protocol; this may be performed before or immediately after trial treatment has been initiated. This CT head scan is part of routine clinical care and is independent of the trial. For patients who are randomised into the trial the scan and results will be used as baseline data.
- ***Clinical***: Patients may need additional head CT scans performed for clinical reasons (e.g., deterioration or stroke recurrence); scans and reports from these will also be collected for the study.

These approaches were used in the ENOS and RIGHT-2 trials

Details of radioactive materials and dose

1 CT Brain – 2 mSv (utilising mean DLP from recent national survey by Public Health England ref. PHE-CRCE-013 (2014) and conversion co-efficient from Shripton et. al. British Journal of Radiology 89 (2016)). This leads to the following maximum protocol dose; 1 CT Head – 2 mSv

Risk Assessment (induction of fatal cancer)

This study requires exposures to ionising radiation. The total protocol dose is 2mSv. This is equivalent to approximately 1 year of background radiation in the UK.

Ionising radiation can cause cancer which manifests itself after many years or decades. The risk of developing cancer as a consequence of taking part in this study is approximately 0.01%. For comparison, the natural lifetime cancer incidence in the general population is about 50%.
The doses involved are too small to cause direct radiation tissue effects such as erythema or epilation.

Clinical Assessment

The scan itself takes about half a minute and does not involve any injections. The scan uses x-rays, which in large amounts can be harmful, but for this extra CT head scan the additional risk to the participant from the scan has been judged to be extremely small.

The objective of the exposure is to assess the extent of the bleeding (haematoma) in the brain to see if it has got worse (larger) or better (smaller) following treatment. An alternative would be MRI brain scan, but this takes longer, and many patients are unsuitable or unable to tolerate it due to claustrophobia.

The procedure for CT and any doses in lay terms are explained in the participant information sheet.

TRANSPORT AND STORAGE OF THE TISSUES

University of Nottingham

Genetic studies

Genetic samples will be taken from participants to help understand the causes of stroke, the relationship with treatment effect and predictors of outcome. Consent will be taken for genetic sampling either at baseline (Day 0) or Day 2. The patient or relative/friend may request destruction of the genetic samples at any time after consent and prior to analysis.

After analysis, all samples will be used to exhaustion or destroyed in accordance with HTA guidance. These tests will be performed for research only and the results will not be fed back to the clinical team.

Local hospital site (Urea and electrolytes, Full blood count)

Blood samples for urea and electrolytes and full blood count will be taken at baseline as part of routine clinical practice and on day 2 after the end of treatment. Samples will be labelled in accordance with local NHS procedures and will be analysed according to the local NHS procedures. These results will be available for the clinical and research teams.

The master database will be held by University of Nottingham on a dedicated research server in a password encrypted file.

LABORATORY ANALYSES

Analysis of routine clinical blood samples will be performed by the recruiting site. Analysis of research blood samples will take place at the University of Nottingham (Division of Clinical Neuroscience).

Blood samples will be collected at the local site as below and processed in accordance with the trial SOPs

After the study ends all samples will be used to exhaustion or destroyed in accordance with HTA guidance. These tests will be performed for research only and the results will not be fed back to the clinical team.

Soluble markers of outcome and efficacy
Several blood biomarkers are surrogate markers of outcome, such as S-100 and neurone specific enolase. Which biomarkers will be assayed will be determined after the trial has finished collecting samples and will be based on the latest scientific knowledge on which biomarkers are the most useful predictors of outcome and efficacy.

Genetic studies
An important aim of the genetic analyses is to determine whether polymorphic differences in candidate genes explain blood pressure and outcome responses to GTN (pharmacogenetic analysis). Which genotyping will be performed will be determined after the trial has finished collecting samples and will be based on the latest scientific knowledge on which analyses are the most useful predictors of outcome and efficacy. Likely analyses will include genes related to the synthesis and metabolism of nitric oxide (e.g., endothelial nitric oxide synthase) and the mechanism of action of antihypertensive agents (e.g., polymorphisms in receptors).

Procedure if blood samples cannot be processed
In circumstances where no appropriately trained members of the research team are available to process blood samples, these tests should be omitted. Participants may still be randomised and treated with the IMP but without these laboratory tests.

STATISTICS and DATA MANAGEMENT PLAN

DATA MANAGEMENT PLAN

This document describes the procedures for the management and assurance of quality of all data collected within a trial throughout the life cycle of the trial from CRF design through to publication of the data, long-term storage, and data sharing. The Sponsor's Standard Operating Procedure will be followed for data management.

General

The Trial Management Committee will include the Trial Programmer and Trial Statistician who are based in the Nottingham Stroke Trials Unit (see Trial Personnel and Contact Details, page 2). The Trial Programmer will design, programme, and maintain the trial system comprising an electronic case report form (eCRF), web front-end, and database. Access to the database will be via an encrypted web application and password access.

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data as an ongoing activity.

Entries on eCRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the trial risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the trial database will be checked. Where corrections are required, these will carry a full audit trail and justification.

Trial data and evidence of monitoring and systems audits will be made available for inspection by the regulatory authority as required.

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Code of Research Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

Data Capture and Data Queries

Data will primarily be captured using electronic CRFs entered through an online web application, with paper versions of the same forms available for completion when required. Instructions for investigators to complete online CRFs will be included in the training slides given at site initiation, with detailed information provided by working practice documents. A demonstration site/database will also be available for investigators to practice using the system.

Data queries will be tracked online by the issue tracker, with warnings shown to investigators and shown on the data queries report. In addition to data queries identified by the trial statistician and programmer, staff in the co-ordinating office can also raise and close issues that they identify.

Protocol violations will be submitted online, primarily by site investigators, and evaluated by the chief investigator who will determine if a serious GCP breach has occurred or not in each case.

Description of Data Entry Validation

The trial programmer will identify required validation for each question on each CRF and it will be applied when each form is submitted, prior to the record being stored. These validation checks will be documented against the CRF itself for use by the trial statistician. The trial statistician will automate additional data checks and submit any exceptions found into the online issue tracker

Data Cleaning and Database Lock

Prior to each database lock, the trial managers/trial co-ordinators will chase outstanding data queries and the lock will take place in accordance with the documented data lock procedure once notification has been given to the trial programmer by the chief investigator. Both interim and final locks will be documented and primarily consist of the creation of a read-only copy of the live database, with each copy available to the trial statistician via the online data extract process.

Monitoring

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data

manipulation. The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data at site up to three times during the period of the study unless issues are highlighted warranting further visits. Monitoring of consent forms and electronic data input will be an ongoing process for the duration of the trial.

STATISTICS

Methods

Data will be analysed by a qualified statistician who is blinded to treatment allocation, using a validated software package. A statistical analysis plan (SAP) will be agreed prior to database lock and release of randomisation codes. The trial will be reported in accordance with CONSORT guidelines including the extension to pilot and feasibility trials, as appropriate.

This is a feasibility trial, and the main analysis will be with descriptive statistics; comparisons between treatment groups will also be performed. Results will be summarised and analysed as follows:

- Counts will be summarised using frequency and percentage (N, %) and compared with binary logistic regression.
- Time to event analyses will be summarised using frequency and percentage (N, %) and compared with Cox proportional hazards regression.
- Ordered categorical variables will be summarised using median [interquartile range] and compared with ordinal logistic regression.
- Continuous variables will be summarised using mean (standard deviation) and compared with multiple linear regression.

We will assess the feasibility of recruiting, treating, and following up patients from 2 centres over three years. We will estimate a recruitment rate, treatment rate and follow-up rate. It is likely that a large definitive trial would be feasible if at least 75 participants were recruited into this study, that compliance with randomised treatment was high and that a high proportion of follow up data was available. Lower recruitment would not preclude progression if there was evidence that the barriers to recruitment identified could be overcome.

Sample size and justification

Since this is a feasibility study with one of the objectives being to determine potential recruitment rates, a formal sample size calculation is not appropriate. If more than 75 participants are randomised from 2 centres over a 31-month period, it is likely that a larger study recruiting approximately 500 participants in around 20 centres would be feasible. Depending on the final sample size calculation for the definitive study, the number of centres and recruitment period could be determined using the information from the rates and patterns observed in the feasibility study.

Assessment of efficacy

This is a feasibility trial and as such will have no formal assessment of efficacy. The proposed primary efficacy outcome in a definitive trial would be death or dependency at day 90, measured using the modified Rankin scale. Shifts in this scale will be summarised for this trial but no formal confirmatory statistical analyses will be performed. Similarly, all other efficacy variables will be summarised using descriptive statistics.

Assessment of safety

Serious adverse events will be summarised using descriptive statistics according to the treatment the participant received.

Procedures for missing, unused and spurious data

Missing data will be reported. The investigation of this data and methods implemented to address the missing data, if appropriate, will be detailed in the SAP.

Definition of populations analysed

All available data will be used including overall numbers of patients presenting in clinic and screening data (where available).

Where summaries by treatment group are provided, these will be based on an intention to treat population i.e., according to the treatment the participant was randomised to, with the exception of safety data.

A safety population will be defined to summarise the safety data in this study. Participants will be summarised according to the treatment they received irrespective of randomisation.

Summaries of the proportion of participants who would form a per protocol population in a larger trial, and reasons for exclusion from a per protocol population will be provided, to allow future planning. No data will be summarised on this population.

ADVERSE EVENTS

Definitions

Adverse events

An adverse event is any unfavourable and unintended sign, symptom, syndrome, or illness that develops or worsens during the period of observation in the study.
An AE does include a / an:

1. Exacerbation of a pre-existing illness.

2. Increase in frequency or intensity of a pre-existing episodic event or condition.

3. Condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.

4. Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

These will not be collected since >4000 patients with hyperacute and acute stroke have been studied in previous trials and GTN has been therapeutically used for >150 years.[49] However, a number of events that may be expected to occur during or shortly after administration of GTN, i.e. an adverse drug reaction, will be collected prospectively:

1. Hypotension requiring intervention (such as raising the legs, administration of intravenous fluids, cessation of open-label hypotensive therapy, or removal of the IMP/sham).

2. Hypertension requiring intervention (such as administration of open-label hypotensive therapy).
3. Headache
4. Infection (such as involving the respiratory or urinary tract).

An AE does not include a / an:

1. Medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that led to the procedure is an AE.

2. Pre-existing disease or conditions present or detected at the start of the study that did not worsen.

3. Situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).

4. Disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.

5. Overdose of concurrent medication without any signs or symptoms.

A **Serious Adverse Event (SAE)** is any adverse event occurring following study mandated procedures, having received the IMP or placebo that results in any of the following outcomes:

1. Death

2. A life-threatening adverse event

3. Inpatient hospitalisation or prolongation of existing hospitalisation

4. A disability / incapacity

5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

All adverse events will be assessed for seriousness, expectedness, and causality:

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

Serious adverse events are common in stroke; a full list of expected SAEs that are not subject to expedited reporting are given in Appendix K.

As the IMP is administered once and has a short half-life, serious adverse events occurring within the first 2 days will be assessed for seriousness, expectedness, and causality. In addition, fatal SAEs will be reported until day 90.

Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to trial treatment administration which makes a causal relationship incompatible or for which other drugs, chemicals or disease provide a plausible explanation**.** This will be counted as "unrelated" for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, but which could also be explained by other drugs, chemicals, or concurrent disease. This will be counted as "related" for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other drugs, chemicals, or concurrent disease. This will be counted as "related" for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

An AE whose causal relationship to the study IMP is assessed by the Chief Investigator as "possible", "probable", or "definite" is an Adverse Drug Reaction.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of adverse events

Participants will be asked to contact the study site immediately in the event of any serious adverse event. All adverse events will be recorded and closely monitored until resolution, stabilisation, or until it has been shown that the study medication or treatment is not the cause. The Chief Investigator (delegated responsibility by the Sponsor) shall be informed immediately (within 24 hours) of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

Where it is the partner of trial participant, consent will be obtained for this observation from both the partner and the medical practitioner.
All serious adverse events will be recorded and reported to the MHRA and REC as part of the annual Development Safety Update Reports. SUSARs will be reported within the statutory timeframes to the MHRA, and REC as stated below. The Sponsor shall ultimately be responsible for adverse event reporting.

Urgent Safety Measures

An Urgent Safety Measure is a procedure taken to protect a research participant when that participant is identified as being at risk of harm in relation to their involvement in a research

project and urgent action, which deviates from the approved protocol, is required to manage the event, and protect the participant.

Any urgent safety measure relating to a Clinical Trial of an Investigational Medicinal Product (CTIMP) should be communicated to the MHRA immediately. They advise that sponsors phone the MHRA Clinical Trial Unit and discuss the event with a safety scientist. The sponsor must then follow-up with notification in writing within three days of the action being taken. The notification should be in the form of a substantial amendment and should describe the event, the measures taken and justification for the measures taken.

SUSARs

A serious adverse event that is either sudden in its onset (anaphylaxis), unexpected in its severity and seriousness or not a known side effect of the IMP *and* related or suspected to be related to the IMP is classed as Suspected Unexpected Serious Adverse Reaction and requires expedited reporting as per the clinical trials regulations.

All serious adverse events that fall or are suspected to fall within these criteria shall be treated as a SUSAR until deemed otherwise.

The event shall be reported immediately (within 24 hours) of knowledge of its occurrence to the Chief Investigator.

The Chief Investigator will:

- Assess the event for seriousness, expectedness, and relatedness to the study IMP.
- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action.
- If the event is deemed a SUSAR, shall, within seven days, enter the required data on the MHRA's eSUSAR web site.
- Shall inform the REC using the reporting form found on the HRA web page within 7 days of knowledge of the event.
- Shall, within a further eight days send any follow-up information and reports to the MHRA and REC.
- Make any amendments as required to the study protocol and inform the ethics and regulatory authorities as required.

Trial Treatment Related SAEs

A serious adverse event that is unexpected in its severity and seriousness *and* deemed directly related to or suspected to be related to the trial treatment but not the IMP shall be reported to the ethics committee that gave a favourable opinion as stated below.

The event shall be reported immediately of knowledge of its occurrence to the Chief Investigator.

The Chief Investigator will:

- Assess the event for seriousness, expectedness, and relatedness to the trial treatment.

- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action.
- If the event is deemed related to the trial treatment shall inform the REC using the reporting form found on the HRA web page within 7 days of knowledge of the event.
- Shall, within a further eight days send any follow-up information and reports to the REC.
- Make any amendments as required to the study protocol and inform the REC as required.

Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator. Should the participant not receive the complete intervention due to, for example, an (serious) adverse drug reaction, they will remain in the study until the end of the trial at day 90 (±30), as completeness of follow-up is essential. However, should they wish to do so, any participant is free to withdraw from the trial at any time and without giving a reason.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The trial will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Medicines and Healthcare products Regulatory Agency (MHRA), Research Ethics Committee (REC), the respective National Health Service (NHS) or other healthcare provider's Research & Development (R&D) department, and the Health Research Authority (HRA) if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the MHRA, R&D and REC are notified as soon as possible, and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, in accordance with the Medicines for Human Use Regulations, Statutory Instrument 2004, 1031 and its subsequent amendments and the UK Department of Health Policy Framework for Health and Social Care, 2017.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant or other legally authorised representative (personal or professional) shall both sign and date the Consent Form before the person can commence follow-up on day 2.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the trial.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial-specific follow-ups will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Drug accountability

Drug supplies will be kept in a secure, limited access storage area under the storage conditions specified by Pharmacy.

The investigator and the local site pharmacist shall maintain records of the study drug's delivery to the pharmacy, an inventory at the site, the distribution to each participant, and the return to the pharmacy or alternative disposition of unused study drugs. These records will include dates, quantities received, batch / serial numbers, expiration dates, and the unique code numbers (patient trial number) assigned to the trial participant. Investigators and /or the local site pharmacists will maintain records that document adequately that the participants were provided with the correct study medication. These records will be part of each patient's Case Report Form (CRF). All study medication packs and bottles received by the pharmacy shall be accounted for.

Case Report Forms

Each participant will be assigned a trial identity code number, (centre number, participant number and initials) allocated at randomisation, for use on CRFs other trial documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy) is entered into the database once for the use of data verification and is not visible when entering trial data.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial in accordance with regulatory requirements and for follow-up as required.
CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled, and dated.
The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and pharmacy records. A CRF may also completely serve as its own source data. Only trial staff as listed on the Delegation Log shall have access to trial documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (MHRA).

DATA PROTECTION

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities (see above). Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one-way encryption method).
Information about the trial in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Trial conduct will be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Trial Delegation Log; CVs of trial staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; drug accountability, pharmacy records and equipment calibration logs.

The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out a site systems audit at least yearly and an audit report shall be made to the Trial Steering Committee.

TRIAL DATA

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data at site up to three times during the period of the study unless issues are highlighted warranting further visits. Monitoring of consent forms and electronic data input will be an ongoing process for the duration of the trial.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the trial risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the trial database will be checked. Where corrections are required, these will carry a full audit trail and justification.

Trial data and evidence of monitoring and systems audits will be made available for inspection by the regulatory authority as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Code of Research Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare. If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this trial will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

Reporting, dissemination, and notification of the results
Trial results will be published in a peer reviewed academic journal. Reporting will be in compliance with CONSORT recommendations. The focus of that article will be to discuss the feasibility of using GTN to treat hyperacute stroke and improve functional outcome. When the study is complete, summary findings will be posted on the support group website. Findings will also be presented at conferences such as UK Stroke Forum, European Stroke Organisation Conference and/or World Stroke Congress annual meeting.

Policy for publication and authorship
The main and secondary trial results will be published by named members of the trial team.

USER AND PUBLIC INVOLVEMENT

The project and protocol were discussed with the Nottingham Stroke Research Partnership Group. The group reviewed the trial design and were highly supportive of the project, including using emergency waiver of consent. The Stroke Research Partnership Group will help with dissemination of the results via the user group website.

STUDY FINANCES

Funding source

This study is funded by NUH Charity, grant code CRF-BATH-NOV 2019 (fund number Q7010).

Participant stipends and payments

Participants will not be paid to participate in the trial. Travel expenses will be offered for any hospital visits in excess of usual care.

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name) _____

Signature: _____

Date: _____

Trial Statistician: (name) _____

Signature: _____

Date: _____

Trial Pharmacist: (name) _____

Signature: _____

Date: _____

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Appendix A. Modified Rankin Scale

All investigators should gain sufficient training and certification to measure mRS.

0 No symptoms at all.
1 No significant disability, despite symptoms; able to carry out all usual duties and activities.
2 Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance.
3 Moderate disability; requiring some help, but able to walk without assistance.
4 Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance.
5 Severe disability; bedridden, incontinent, and requiring constant nursing care and attention.
6 Dead.

Score 1 to 6. Death is assigned 6.

See [50,51]

Appendix B. Glasgow Coma Scale

Eye movement

1 = None
2 = To pain
3 = To speech
4 = Spontaneous

Verbal response

1 = None
2 = Incomprehensible
3 = Inappropriate
4 = Confused
5 = Orientated

Motor response

1 = None
2 = Extension
3 = Flexor response
4 = Withdrawal
5 = Localises pain
6 = Obeys commands

Total score out of 15 (range 3 – 15). Death is assigned 2.

See [52]

Appendix C. National Institutes of Health Stroke Scale

All investigators should gain sufficient training and certification to measure NIHSS.

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort). (Please also see http://www.ninds.nih.gov/doctors/NIH_Stroke_Scale.pdf for pictures associated with this score)

1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.

0 = **Alert;** keenly responsive.

1 = **Not alert**; but arousable by minor stimulation to obey, answer, or respond.

2 = **Not alert**; requires repeated stimulation to attend or is obtunded and requires strong or painful stimulation to make movements (not stereotyped).

3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.

1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.

0 = **Answers** both questions correctly.

1 = **Answers** one question correctly.

2 = **Answers** neither question correctly.

1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.

0 = **Performs** both tasks correctly.

1 = **Performs** one task correctly.

2 = **Performs** neither task correctly.

2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.

0 = **Normal.**

1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes but forced deviation or total gaze paresis is not present.

2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic manoeuvre.

3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.

0 = **No visual loss.**

1 = **Partial hemianopia.**

2 = **Complete hemianopia.**

3 = **Bilateral hemianopia** (blind including cortical blindness).

4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.

0 = **Normal** symmetrical movements.

1 = **Minor paralysis** (flattened nasolabial fold, asymmetry on smiling).

2 = **Partial paralysis** (total or near-total paralysis of lower face).

3 = **Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face).

5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.

0 = **No drift;** limb holds 90 (or 45) degrees for full 10 seconds.

1 = **Drift;** limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.

2 = **Some effort against gravity;** limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.

3 = **No effort against gravity;** limb falls.

4 = **No movement.**

UN = **Amputation** or joint fusion, explain: _____

5a. Left Arm

5b. Right Arm

6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.

0 = **No drift;** leg holds 30-degree position for full 5 seconds.

1 = **Drift;** leg falls by the end of the 5-second period but does not hit bed.

2 = **Some effort against gravity;** leg falls to bed by 5 seconds but has some effort against gravity.

3 = **No effort against gravity;** leg falls to bed immediately.

4 = **No movement.**

UN = **Amputation** or joint fusion, explain: _____

6a. Left Leg

6b. Right Leg

8. **Limb Ataxia:** This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.

In case of blindness, test by having the patient touch nose from extended arm position.
0 = **Absent.**
1 = **Present in one limb.**
2 = **Present in two limbs.**
UN = **Amputation** or joint fusion, explain: _____

8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.

0 = **Normal;** no sensory loss.

1 = **Mild-to-moderate sensory loss;** patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched.
2 = **Severe to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.

9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.
0 = **No aphasia;** normal.
1 = **Mild-to-moderate aphasia;** some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response.
2 = **Severe aphasia;** all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response.
3 = **Mute, global aphasia;** no usable speech or auditory comprehension.

10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.

0 = **Normal.**

1 = **Mild-to-moderate dysarthria;** patient slurs at least some words and, at worst, can be understood with some difficulty.

2 = **Severe dysarthria;** patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia or is mute/anarthric.

UN = **Intubated** or other physical barrier,
explain:_____

11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.

0 = **No abnormality.**

1 = **Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities.

2 = **Profound hemi-inattention or extinction to more than one modality;** does not recognize own hand or orients to only one side of space.

Score out of 42 (range 0-42). Death is assigned +43.

See [53]

Appendix D. Barthel Index

Task	Criteria	Score
Bowels	Incontinent	0
	Occasional accident (once per week)	5
	Continent	10
Bladder	Incontinent, or catheterised and unable to manage alone	0
	Occasional accident (maximum once per 24 hours)	5
	Continent	10
Grooming	Needs help with personal care	0
	Independent face/hair/teeth/shaving (implements provided)	5
Toilet use	Dependent	0
	Needs some help, but can do something alone	5
	Independent (on and off, dressing, wiping)	10
Feeding	Unable	0
	Needs help cutting, spreading butter, etc.	5
	Independent	10
Transfer (bed to chair and back)	Unable, no sitting balance	0
	Major help (one or two people, physical), cab sit	5
	Minor help (verbal or physical)	10
	Independent	15
Mobility	Immobile	0
	Wheelchair independent, including corners	5
	Walks with help of one person (verbal or physical)	10
	Independent (but may use any aid: for example, stick)	15
Dressing	Dependent	0
	Needs help but can do about half unaided	5
	Independent (including buttons, zips, laces, etc.)	10
Stairs	Unable	0
	Needs help (verbal, physical, carrying aid)	5
	Independent	10
Bathing	Dependent	0
	Independent (or in shower)	5

Score out of 100 (range 0-100). Death is assigned -5.

See [54,55]

Appendix E. EuroQoL, EQ-5D

Score 1 – 3 for each group/dimension.

Group 1
1. I have no problems in walking about
2. I have some problems in walking about
3. I am confined to bed

Group 2
1. I have no problems with self-care
2. I have some problems with washing or dressing
3. I am unable to wash or dress myself

Group 3
1. I have no problems performing my usual activities (e.g., work, study, housework, family, or leisure activities
2. I have some problems performing usual activities
3. I am unable to perform my usual activities

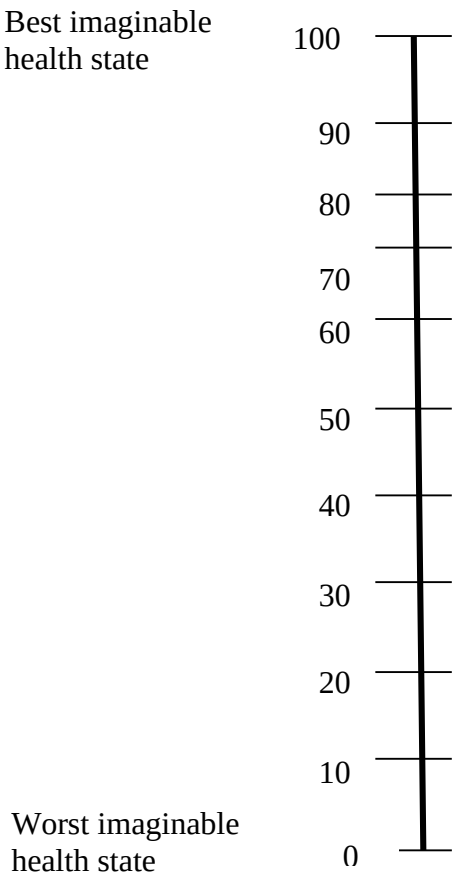
Group 4
1. I have no pain or discomfort
2. I have moderate pain or discomfort
3. I have extreme pain or discomfort

Group 5
1. I am not anxious or depressed
2. I am moderately anxious or depressed
3. I am extremely anxious or depressed

The Health Utility Scale (HUS) is derived from the EQ-5D. Death is assigned 0.

See [56,57]

Health state today by visual analogue scale (best imaginable to worst imaginable)



Score single integer between 0 and 100. Death is assigned -1.

See [58]

Appendix F – Adult lifestyles and function interview (ALFI) - MMSE

QUESTIONS	Maximum score	Patient's score
What is the year/month/day/date/time?	5	
Where are we now – country/town/district/building?	4	
I am going to name three objects and I want you to repeat them after me: apple, table and coin. Please repeat them.	3	
Can you subtract 7 from 100 (93, 86, 79, 72, 65)?	5	
Can you recall the three words I asked you to remember?	3	
Can you repeat "No ifs, ands or buts"?	1	
What is the thing called that you are speaking into as you talk to me?	1	
Total score	22	

Appendix G. Cognitive Testing

TICS-M

Please note that this test is designed for telephone use. In the event follow up is done in person the entire test must be completed verbally, i.e., the memory words must not be shown to the patient.

Orientation:

1(a). What day of the week is it?	Day	☐
(b). What is today's date?	Date	☐
	Month	☐
	Year	☐
(c). What season are we in?	Season	☐
2. What is your age?	Age	☐
3. What is your telephone number?	Correct	☐

Registration/ Free Recall:

1. I am going to read you a list of 10 words. Please listen carefully and try to remember them. When I am done, tell me as many as you can in any order. Ready?

Cabin	☐
Pipe	☐
Elephant	☐
Chest	☐
Silk	☐
Theatre	☐
Watch	☐
Whip	☐
Pillow	☐
Giant	☐

Now tell me the words you can remember

Attention/Calculation:

5. Please take away 7 from 100	93	☐
Now continue to take 7 away from what	86	☐
you have left over until I ask you to stop	79	☐
	72	☐
	65	☐
6. Please count backwards from 20 to 1	No mistakes	☐

Comprehension, Semantic and Recent Memory:

7. What do people usually use to cut paper?	Scissors	☐
8. What is the prickly green plant found in the desert?	Cactus	☐
9. What is the name of the reigning monarch?	Correct Name	☐
10. What is the opposite direction to east?	West	☐
11. What is the surname of the prime minister?	Correct Name	☐

Language/Repetition:

12. Please listen carefully and repeat this: "Methodist episcopal"	Exactly right	☐

Delayed Recall:

13. Please repeat as many of the 10 words I asked you to remember earlier

- Cabin ☐
- Pipe ☐
- Elephant ☐
- Chest ☐
- Silk ☐
- Theatre ☐
- Watch ☐
- Whip ☐
- Pillow ☐
- Giant ☐

Score 1 point for each correct answer.

Score _____

Score out of 39 (range 0-39). Death is assigned -1.

See [59]

Concentration (from MMSE) Spell WORLD backwards (or language specific equivalent)

Score out of 5

Verbal Fluency

Now you have 1 minute to name as many animals as you can think of. Ready? Start now!

Write down each word and score 1 mark for each animal named. Do not score repetitions.

Score 0, 1, 2, 3, 4, 5 etc. Death is assigned -1.

See [60]

Appendix H. Zung Depression Rating Scale (short)

With scores:	Seldom or never	Some of the time	Good part of time	Most of time
I feel downhearted and blue	1	2	3	4
I have trouble sleeping at night	1	2	3	4
Morning is when I feel best	4	3	2	1
I can eat as much as I used to	4	3	2	1
I get tired for no reason	1	2	3	4
I find it difficult to make decisions	1	2	3	4
I feel hopeful about the future	4	3	2	1
I feel that I am useful and needed	4	3	2	1
My life is somewhat empty	1	2	3	4
I still enjoy the things I used to do	4	3	2	1

Short Zung IDS Index = 100 x Total / 40. Depression => 70. Death is assigned 102.5.

See [54,61,62]

Appendix I. Frailty score on baseline neuroimaging

Component items: previous infarct (cortical or subcortical), atrophy, white matter changes

None	No items present
Mild-moderate	1-2 items present
Severe	All items present

Score out of 2 (range 0-2). Death is assigned 3.

See [63]

Appendix J. Small vessel disease score on baseline neuroimaging

Component items: white matter hyperintensities (graded), lacunes

None	No items present
Mild-moderate	Mild-moderate WMH and/or lacune(s)
Severe	Severe WMH and lacune(s)

Score out of 2 (range 0-2). Death is assigned 3.

See [63]

Appendix K: Expected events not subject to expedited reporting

The following events are expected with administration of glyceryl trinitrate and are, therefore, not subject to expedited reporting:

- Headache
- Hypotension
- Allergic skin reactions
- General allergic reactions

All of the events above are listed in the GTN summary of product characteristics.

After stroke the following events are expected and therefore not subject to expedited reporting"

Cardiovascular

Acute coronary syndrome (ACS)
Angina: stable
Angina: unstable (UA)
Angina: type undefined
Atrial fibrillation (AF) or atrial flutter
Bradycardia
Cardiac (mural) thrombus
Cardiac dysrhythmia
Cardiac failure
Carotid dissection
Carotid stenosis: carotid endarterectomy
Carotid stenosis: no carotid endarterectomy
Carotid stenosis: carotid stenting
Chest pain (NOT cardiac)
Collapse
Deep vein thrombosis (DVT)
Endocarditis
Heart failure
Hypertension
Hypotension
Left atrial myxoma
Left ventricular failure (LVF)
Myocardial Infarction: NSTEMI
Myocardial Infarction: STEMI
Myocardial Infarction: type undefined
Patent foramen ovale (PFO)
Pericardial bleed
Peripheral Arterial Disease (PAD) / ischaemic limb

Sudden Cardiac Death (SCD)
Supra-ventricular Tachycardia
Systemic embolism
Tachycardia
Vasovagal episode
Vascular event (not otherwise specified)
Ventricular tachycardia (VT)

Nervous System
Agitation
Alzheimer's disease (AD)
Anxiety / apprehension
Brain Tumour: primary
Brain Tumour: secondary
Cerebral oedema
Cognitive decline
Complication of initial stroke
Cortical vein thrombosis
Deafness / hearing loss
Dementia: type undefined
Depression
Dizziness
Dysarthria
Dysphagia
Dysphasia
Extension of initial ischaemic stroke
Extradural haematoma / bleed (EDH)
Extraspinal bleed
Functional / Mimic / Pseudo Stroke
Haemorrhagic stroke - primary haemorrhage/bleed
Haemorrhagic transformation of infarction (HTI): HT1 or HT2
Haemorrhagic transformation of infarction (HTI): HT1
Haemorrhagic transformation of infarction (HTI): HT2
Haemorrhagic transformation of infarction (HTI): PH1 or PH2
Haemorrhagic transformation of infarction (HTI): PH1
Haemorrhagic transformation of infarction (HTI): PH2
Haemorrhagic transformation of infarction (HTI): type undefined
Hallucinations
Headache
Hydrocephalus
Intracranial aneurysm
Intraspinal bleed / haematoma

Intraspinal infarct
Ischaemic stroke: no blood
Ischaemic stroke with HTI: HT1 or HT2
Ischaemic stroke with HTI: HT1
Ischaemic stroke with HTI: HT2
Ischaemic stroke with HTI: PH1 or PH2
Ischaemic stroke with HTI: PH1
Ischaemic stroke with HTI: PH2
Migraine
Nerve Entrapment
Neurological deterioration
Neurological Event: NOT stroke/TIA
Stroke: type undefined
Sedation
Seizure / Convulsions
Sensory loss
Stroke type: type unknown
Subarachnoid haemorrhage (SAH)
Subdural haematoma / bleed (SDH)
Transient Ischaemic Attack (TIA) - imaging negative
Transient Ischaemic Attack (TIA) - imaging positive
Transient Ischaemic Attack (TIA) - no imaging
Vascular dementia (VaD)
Vertigo
Visual loss
Weakness

Respiratory

Acute respiratory failure: type 1
Acute respiratory failure: type 2
Acute respiratory failure: type undefined
Asthma
Bronchitis
Bronchospasm
Chest infection
Chronic obstructive pulmonary disorder (COPD)
Chronic obstructive pulmonary disorder (COPD): exacerbation
Epistaxis
Emphysema
Haemoptysis
Hypoxia
Interstitial pneumonitis

Pleural effusion
Pneumonia
Pneumothorax
Carcinoma: primary lung
Pulmonary embolism (PE)
Pulmonary fibrosis
Pulmonary haemorrhage
Respiratory tract infection, lower (LRTI)
Respiratory tract infection, upper (URTI)
Secondary lung cancer
Shortness of breath

Gastro-Intestinal
Abdominal pain
Bowel ischaemia
Carcinoma: bowel
Cholecystitis
Colitis
Constipation
Diarrhoea
Diverticulitis
Dysphagia
Gall stones
Gastroenteritis
Gastrointestinal disturbance
Gastrointestinal infarction
Haemorrhoids: bleeding
Haematemesis
Heartburn
Hepatitis
Hernia
Incontinence: faecal
Liver/hepatic impairment/dysfunction
Lower GI bleed
Melaena
Nausea
Oesophagitis
Oral ulceration
Pancreatitis
Peptic Ulcer
Carcinoma: primary liver
Rectal bleed

Liver metastasis
Stomatitis
Upper GI bleed
Vomiting
Weight loss

Genito-Urinary
Carcinoma: bladder
Glomerulonephritis
Haematuria
Incontinence: urinary
Kidney stones
Penile bleed
Carcinoma: renal primary
Prostate cancer
Renal cyst
Kidney/renal impairment/failure/disease: acute (ARF)
Kidney/renal impairment/failure/disease: chronic (CKD)
Kidney/renal impairment/failure/disease: undefined
Sexual dysfunction
Urinary retention
Urinary tract infection (UTI)
Vaginal bleed

Haematological
Acquired haemophilia
Agranulocytosis/granulocytopenia
Anaemia: type undefined
Anaemia: aplastic
Anaemia: microcytic
Anaemia: macrocytic
Haemoglobin drop: asymptomatic
Haemoglobin drop: dilutional
Haemoglobin drop: unidentified
Eosinophilia
Hypersensitivity including oropharyngeal swelling, urticaria, angioedema etc.
Leukopenia
Lymphadenopathy
Methaemoglobinaemia
Neutropenia
Pancytopenia
Polycythaemia

Thrombocytopenia
Thrombotic thrombocytopenic purpura (TTP)
Vasculitis

Immunological
Haematological
Allergic reaction
Anaphylactic reaction
Hypersensitivity

Metabolic / Endocrine
Acid base disturbance
Dehydration
Diabetes mellitus (Type II)
Electrolyte disturbance
Hyperglycaemia
Hyperthyroidism
Hyperuricaemia
Hypoglycaemia
Hypothyroidism

Musculoskeletal / Cutaneous
Arthritis / arthralgia
Bleed: gingival
Bleed: skin
Bruising, ecchymoses
Bullous dermatitis
Cellulitis
Cramps
Eczema
Flushing
Fractured bone
Gout
Hypersensitivity
Intra-articular bleed, haemarthrosis
Intramuscular bleed with compartment syndrome
Intramuscular bleed without compartment syndrome
Muscle twitching