Study Title: MINOcycline in the Treatment of Atheroma that are Unstable or Ruptured Study (MINOTAUR)

Ethics Ref: 269021

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AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1.2	14/06/2019	NRE	1. Addition of exclusion criteria related to minocycline contraindications/interactions. 2. Addition of section 5.3 (Interim analysis plan).
2	1.3	31/07/2019	NRE	1. Change to Chief Investigator. 2. Change of "Addenbrooke's" to "Cambridge University Hospitals." 3. Inclusion of insurance statement.
3	1.4	11/03/2020	NRE	Amendments following peer review: 1. Specification of eGFR method. 2. Addition of blood test information. 3. Revised sample size text.
4	1.5	07/12/2020	NRE	1. Reduction in stenosis degree to 30% if felt to be causative. 2. Increase in minimum age to 50 years based on Nuclear Medicine review.
5	1.6	14/12/2020	NRE	1. Cycle Pharmaceuticals changed to Varsity Pharmaceuticals. 2. "12 week" follow-up amended to "90 days." 3. Amended to make CUH sole sponsor.
6	1.7	27/02/2021	NRE	Amendment for individuals who lose capacity during the

				study (as per REC review).

Synopsis

Study Title	MINOcycline in the Treatment of Atheroma that are Unstable or Ruptured (MINOTAUR) study.
Internal ref. no.	
Study Design	Single centre, interventional pilot study.
Study Participants	Individuals with an ischaemic stroke due to ipsilateral carotid stenosis of greater than 30% that is felt to be causative.
Planned Sample Size	24 (12 in each of the intervention and standard care cohorts).
Follow-up duration	90 days.
Planned Study Period	Two years.
Primary Objective	To investigate the natural history of microcalcification in symptomatic carotid atherosclerosis and whether this is altered by minocycline.
Secondary Objectives	1. To measure change in microcalcification activity in non-culprit carotid atheroma in those receiving minocycline compared to 'standard care.' 2. To measure biomechanical stress in symptomatic and non-culprit arteries in relation to NaF TBR_{max}. 3. To measure change in macrocalcification (as measured using Agatston scoring) in the aortic arch, and symptomatic and non-culprit carotid arteries in the minocycline-treated group and 'standard care.'
Primary Endpoint	NaF uptake (expressed as most diseased segment TBR_{max})
Secondary Endpoints	1. NaF TBR_{max} in non-culprit carotid atheroma. 2. Biomechanical stress in culprit and non-culprit arteries. 3. Agatston scoring of culprit and non-culprit carotid arteries, and aortic arch.
Intervention (s)	Minocycline 100mg twice daily in addition to best medical therapy compared to 'standard care' only.

1. BACKGROUND AND RATIONALE

1.1 Background

Stroke is a leading cause of adult disability in the United Kingdom, putting a major burden on patients, carers, healthcare, and society. A major cause of stroke is large artery atherosclerosis (literally “hardening of arteries”), estimated to underlie between 15% to 25% of ischaemic strokes [1, 2], and consequently the carotid arteries represent one of the most dangerous few centimetres in the human body. Atherosclerosis involves the development of lipid-rich atheroma (also termed “plaques”) in response to vascular risk factors, including age, male sex, ethnicity, hyperlipidaemia, hypertension, smoking, and diabetes mellitus [3]. Carotid atherosclerosis will typically occur on the background of systemic atherosclerosis, with concomitant disease within the coronary arteries estimated to occur in 28-58% of asymptomatic individuals [4-6].

1.2 The inflammatory basis of atherosclerosis and the “vulnerable plaque” hypothesis

Atherosclerosis is an inflammatory disease where the accumulation of oxidised lipids prompts an inflammatory response in the vessel wall through pro-inflammatory mediators (Ross 1999). Plaque rupture, and the consequent thromboembolic events that trigger a stroke or transient ischaemic attack (TIA), is triggered by two complementary processes: enzymatic degradation and mechanical disruption of the fibrous cap overlying the atheroma. The fibrous cap is composed of triple-helical collagen, where collagen formation is promoted by vascular smooth muscle cells (VSMCs) in a dynamic process in response to cytokine signaling [7, 8]. Atheromatous plaques have been found to be associated with increased levels of metalloproteinases MMP-1 and MMP-13 [9], MMP-8 [10] and MMP-1, MMP-2 and MMP-3 [11]. In these studies, increased MMP levels were associated with increased collagenolysis and thinner fibrous caps. The increased levels of MMPs have been co-localised to plaque “shoulders,” the most vulnerable regions of the atheroma to rupture, and around the lipid core itself [9, 11].

Mechanical destabilisation is caused by microcalcium deposits; calcium deposits smaller than 50 μm as detected by *ex vivo* CT [12]. These deposits represent both a consequence and precipitant of inflammation, and may either contribute to plaque rupture or coalesce to form protective macrocalcification [13] (**Figure 1**).

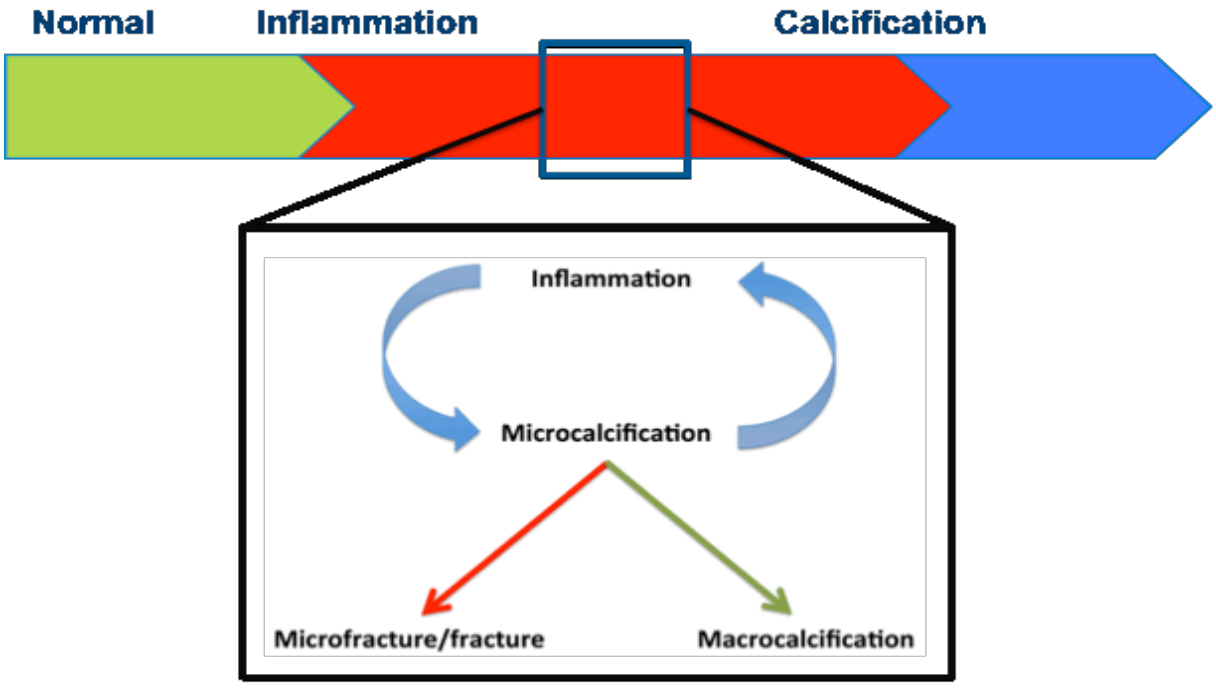


Figure 1: The natural history of atherosclerosis. Schema of the relationship of inflammation and microcalcification in the natural history of atherogenesis.

The inflammatory focus within the necrotic core promotes an osteogenic environment that affects cellular transdifferentiation. VSMCs are derived from the same pluripotent mesenchymal cell as osteoblasts and can undergo osteogenic transformation. A small study of coronary atheroma suggested that microcalcification may increase the circumferential stress in regions of a thin fibrous cap with pre-existing circumferential stress by up to a factor of two [14]. Subsequent work showed that this mechanical disruption of the fibrous cap is location specific, with microcalcification only seen to cause plaque rupture when it occurred within a region of pre-existing high background stress and in fibrous caps thinner than 80 μm, as well as being affected by the shape of the microcalcification. Although spherical microcalcification increased circumferential stress by a factor of two as previously reported, elongated microcalcifications caused even higher stress increases [15]. A larger study of 107 coronary vessels has supported these findings, indicating that that tissue stress may increase up to fivefold in the presence of concentrated microcalcification, and that stress on thin fibrous caps with no microcalcification (107 kPa) fell below the threshold believed to be necessary for rupture (300 kPa) [12, 16]. The authors conclude that although a thin cap may reach the rupture threshold with a single microcalcification, a clustering of a sufficient number of microcalcifications may be enough to exceed the vulnerability threshold [12].

1.3 ^{18}Fluoride-sodium fluoride positron emission tomography for detection and quantification of microcalcification:

The small size of microcalcification means that conventional clinical imaging (computed tomography, CT, and magnetic resonance imaging, MRI) is unable to detect their accumulation *in vivo*. One solution to this problem is the use of 18Fluoride-sodium fluoride (NaF) positron emission tomography (PET). NaF is a radioligand that has been used for bone imaging since 1962, finding clinical use in the PET evaluation of osseous metastatic disease over the subsequent decades [17]. NaF identifies sites of active microcalcification, where radiolabelled fluoride is exchanged for the hydroxyl group in hydroxyapatite to form fluoroapatite [18].

Vascular uptake of NaF was first investigated by Derlin et al. where incidental arterial uptake was detected in 57 (76%) of 75 asymptomatic individuals undergoing whole-body NaF-PET for assessment for bone metastases. This retrospective study found 254 sites of uptake across all arterial territories (common carotid arteries, thoracic and abdominal aorta, iliac arteries, and femoral arteries) in these individuals, but although NaF uptake was more likely to occur in lesions with extensive macrocalcification, there was no statistically significant association between NaF maximum standardised uptake values (SUV_{max}) and calcium score using an ordinal 0-4 scale. Furthermore, distinct areas of NaF uptake and macrocalcification occurring in isolation suggested NaF uptake reflected the active mineralisation process in microcalcification rather than simply the burden of macrocalcification [19].

Further examples of a lack of co-localisation between regional macrocalcification and regional NaF uptake have been found in other asymptomatic cohorts, with an inverse relationship between NaF tissue-to-background ratio (TBR) and semi-quantitative plaque calcium density across all arterial territories reported by Fiz et al. [20, 21]. Morbelli et al. demonstrated that the presence of cardiovascular risk factors (as measured by a Framingham risk score) correlated with NaF uptake (both SUV_{max} and TBR_{max}) but not arterial macrocalcification (measured using Agatston scoring), through the study's measurement of uptake across the whole vessel may miss focal concomitant areas of macrocalcification and NaF uptake [21].

Further studies have found strong associations between carotid NaF uptake and calcification and the presence of cardiovascular risk factors in a neurologically asymptomatic oncologic population. In one study, Derlin et al. found 140 (26%) of 538 carotid artery segments had both NaF uptake and macrocalcification detectable by CT, and 46 (8.6%) had macrocalcification but no NaF uptake [22]. In contrast to the results of the aforementioned studies, there were no areas of NaF uptake without visible macrocalcification. In this asymptomatic population, NaF SUV_{max} correlated strongly with the extent of calcification within the carotid plaque (measured as an ordinal scale according to proportion of arterial wall circumference calcified) ($r=0.85$, $p<0.0001$). As arterial macrocalcification reflects the vascular risk profile [23], it is unsurprising that the same study found associations between NaF SUV_{max} and some cardiovascular risk factors; age, male sex, hypertension, and hypercholesterolaemia. However, there was no association with previous cardiovascular events, although associations of NaF uptake with a history of smoking or diabetes approached, but did not reach, significance ($p=0.12$ and $p=0.11$ respectively). Tracer accumulation weakly correlated inversely with BMI ($r =-0.13$, $p=0.04$). In contrast, the presence of plaque macrocalcification was significantly associated with age, male sex, hypertension, hypercholesterolaemia, diabetes, history of smoking, and prior cardiovascular events. Finally, individuals with multiple cardiovascular risk factors had a higher proportion of plaques with NaF uptake as a dichotomous measure, though there was no significant correlation between quantified NaF uptake and the number of cardiovascular risk factors.

In addition to this relationship between NaF uptake and macrocalcification, dual-tracer studies have elucidated the relationship between different metabolic processes within an asymptomatic cohort. Derlin et al. performed a dual-tracer PET/CT study using FDG-PET and NaF-PET in a further asymptomatic oncological patient cohort and found that of 215 arterial lesions identified by either tracer (across aorta, carotid, iliac, and femoral arteries), only in 14 (6.5%) was there concomitant FDG and NaF uptake. These results imply that macrophage-driven inflammation and microcalcification are two related but distinct processes. Furthermore, whilst 77.1% of NaF-positive plaques co-localised with macrocalcification, only 14.5% of FDG-positive plaques co-localised with macrocalcification [24]. In contrast to their previous study, in this study Derlin et al. found a moderate correlation between NaF SUV_{max} and calcification score ($r=0.36$, $p<0.001$).

While oncological cohorts have indicated the ability of NaF-PET to identify plaques in asymptomatic disease, there has been increasing attention to the use of the tracer to identify high vulnerability plaques. In 2012, Dweck et al. were the first to assess disease within the coronary arteries when they performed a prospective dual tracer (FDG and NaF) study in individuals with and without coronary artery disease (CAD, the presence of which was defined as a previous clinical diagnosis or having a coronary calcium score above zero) [25]. Unlike the ubiquitous myocardial uptake of FDG, NaF showed a better signal-to-noise ratio and its uptake was quantifiable in 96% of the coronary territories examined. This study had a small control group (thirteen participants) but was able to demonstrate NaF uptake to be higher in those with coronary artery disease than the control group (TBR_{max} 1.64±0.49 versus 1.23±0.24 respectively; p=0.003). As was observed in asymptomatic cohorts, the authors noted focal NaF uptake both overlying and distinct from areas of macrocalcification, as well as areas of macrocalcification with no tracer uptake. Of participants with the highest level of macrocalcification (coronary Agatston scores >1,000), only 59% showed significant NaF uptake above that of controls. However, overall within plaques there was a strong correlation between coronary calcification score and NaF uptake (r=0.652, p<0.001). In individuals with multiple coronary plaques, culprit plaques had an average 50% increase in NaF uptake compared to inactive plaques (2.14±0.42 versus 1.43±0.32 respectively; p<0.001). Finally, NaF uptake reflected both disease severity (proportion with angina symptoms, need for prior revascularisation, and previous major adverse cardiac events) and cardiovascular risk burden (Framingham risk scores), where uptake was significantly higher.

The above study largely considered those with atherosclerosis, but not necessarily after acute events. Joshi et al. compared NaF uptake in individuals recruited prospectively with recent myocardial infarction (both ST-segment and non-ST-segment elevation) and stable angina. They showed increased tracer uptake is associated with symptomatic coronary plaques: in those with myocardial infarction, the average TBR_{max} of the culprit plaque (1.66; SD 1.4-2.25) was higher than elsewhere in the coronary vasculature (1.24; SD 1.06-1.38) (p<0.0001). It is interesting to note that three individuals who had a myocardial infarction showed no NaF uptake, and of those two were younger with only mild irregularities on angiography, suggesting that these events may be triggered by thrombosis from plaque erosion rather than plaque rupture. Supporting this, those with NaF positive lesions (those with a TBR more than 25% than a proximal reference lesion) had higher concentrations of plasma troponin at baseline (median 3.35 [IQR 2.35-10.2] versus 2.45 [1.85-4.02] ng/L; p=0.047), implying a role for plaque erosion or subclinical rupture in the NaF negative lesions

[26]. It is also worth noting that of NaF positive plaques, only 28% were obstructive on coronary angiography (>70% luminal stenosis).

In the same study, increased NaF uptake was also seen in morphologically high-risk but unruptured plaques seen on intravascular ultrasound (greater positive remodeling, greater microcalcification, and larger necrotic core) and CT. This implies that NaF uptake reflects the microcalcification process rather than increased surface area following plaque rupture [26]. Similar results were seen in another study across 123 coronary atherosclerotic lesions assessed by CT [27].

Recent studies of carotid atheroma in transient ischaemic attacks and minor strokes have found increased NaF uptake in culprit atheroma [28, 29]. Our recent pilot work demonstrates that uptake of NaF in symptomatic culprit atheroma is increased compared to the non-culprit asymptomatic plaque (**Figure 2, Table 1, Figure 3**). It is the largest symptomatic stroke cohort investigated with NaF-PET/CT to date and is the first to have been conducted including a range of stroke severities.

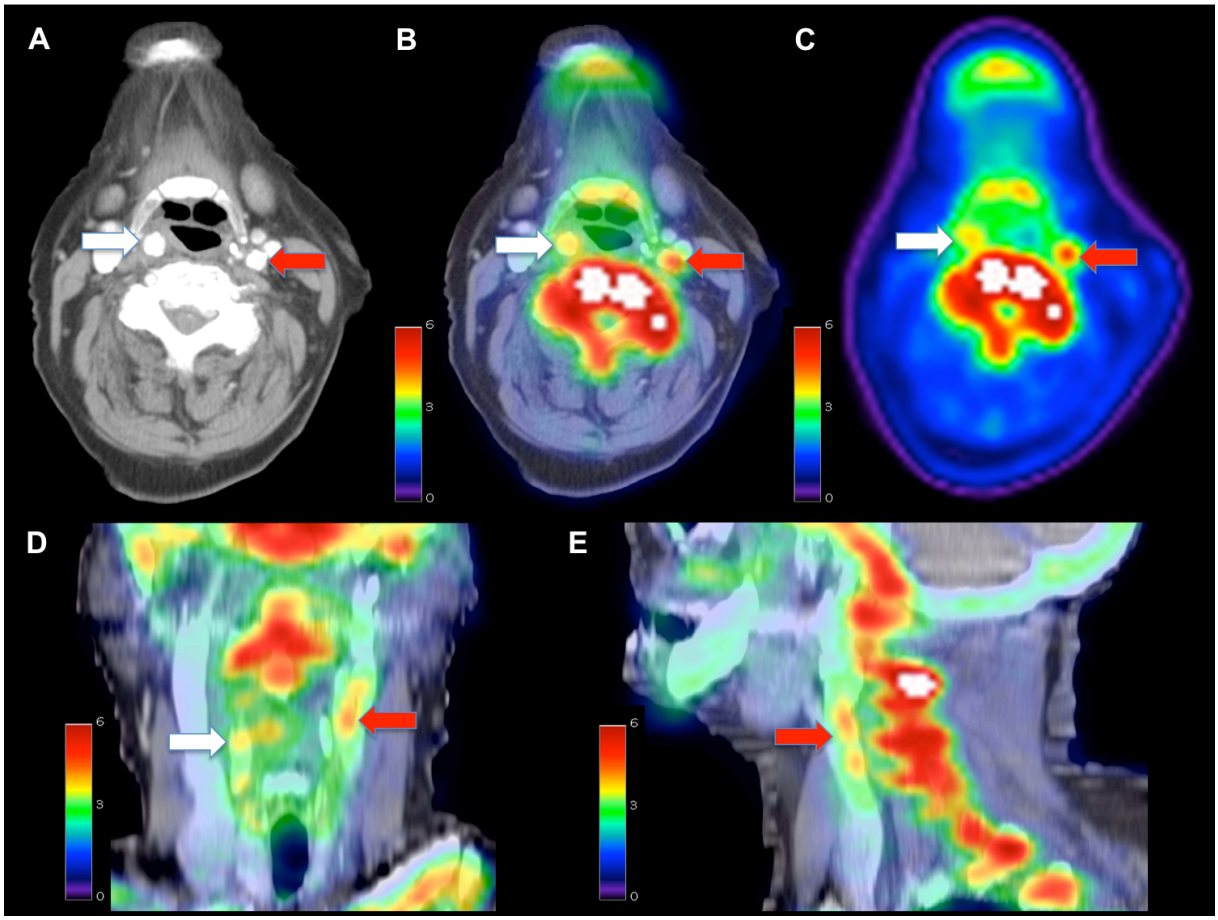


Figure 2: NaF uptake in symptomatic disease. (a) axial CT angiogram, (b) axial NaF-PET/CT, (c) axial PET, (d) coronal NaF-PET/CT, (e) sagittal NaF-PET/CT showing an asymptomatic right carotid artery (white arrow) and a symptomatic left carotid artery (red arrow).

	Culprit carotid artery	**Non-culprit carotid artery**	**Significance**
Median MDS TBR_{max} (IQR)	2.68 (IQR 0.63)	2.39 (IQR 1.02)	p<0.001
Median WV TBR_{max} (IQR)	1.85 (IQR 0.28)	1.79 (IQR 0.60)	p=0.10

Table 1: NaF TBR_{max} readings for symptomatic and asymptomatic carotid arteries (MDS = most diseased segment, WV = whole vessel).

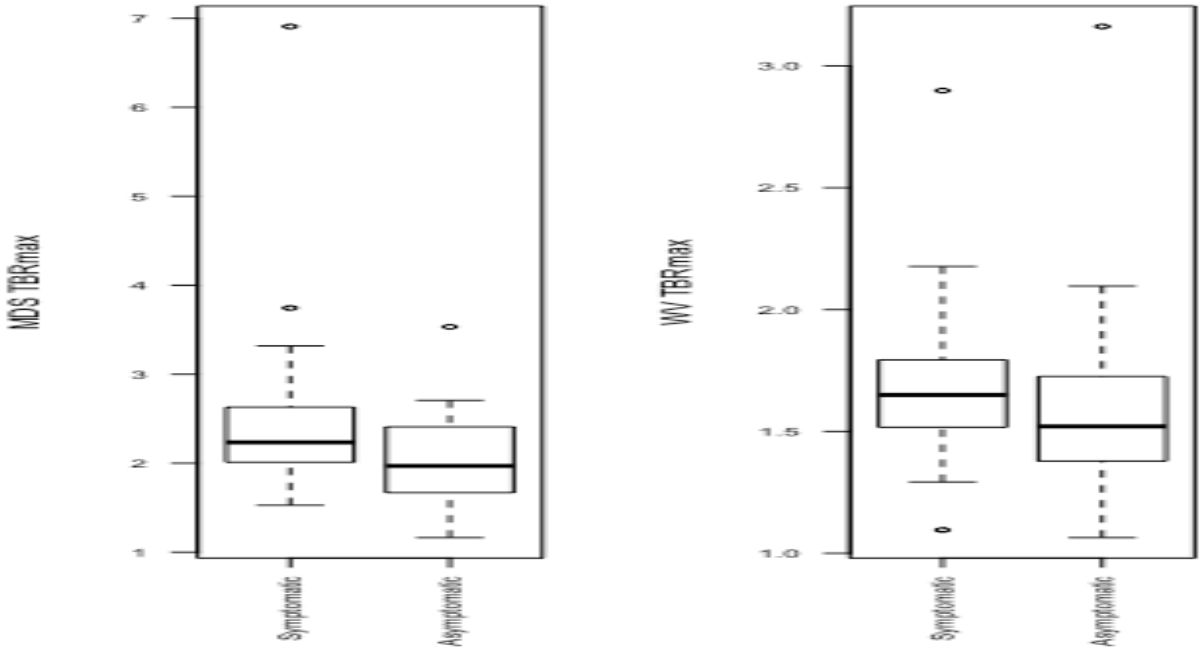
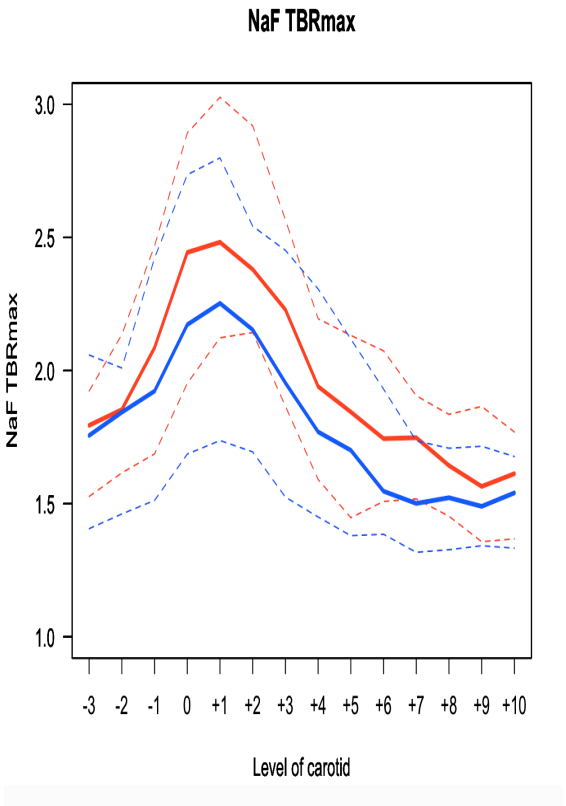


Figure 3: NaF TBR_{max}. Boxplots showing TBR_{max} between symptomatic and asymptomatic arteries for MDS (left), and WV (right) TBR_{max}.



Furthermore, the pattern of NaF uptake suggests that microcalcification is a focal disease, with focal measures of tracer uptake (MDS TBR_{max}) differing significantly between symptomatic and asymptomatic sides. However, the average TBR_{max} readings for the entire artery (WV TBR_{max}) were not significantly different between symptomatic and asymptomatic sides (**Figure 4**).

Figure 4: Composite NaF TBR spatial distributions. Comparison of NaF TBR_{max} at each slice along the symptomatic (red line) and asymptomatic (blue line) artery (upper and lower interquartile limits shown by dashed lines).

Our results have also demonstrated excellent inter-rater reproducibility of NaF-PET/CT, with an intra-class correlation coefficient for TBRmax of 0.96, consistent with previously reported values of 0.99 [25].

1.5 The effect of minocycline on microcalcification:

Minocycline is an oral antibiotic used for acne vulgaris. It is a member of the tetracycline class of antibiotics. Minocycline was patented in 1961 and came into commercial use in the 1970s.

Professor Melinda Duer of the Department of Chemistry, University of Cambridge, and Professor Cathy Shanahan of King's College London have led the academic discussion of the extent of the cellular and extra-cellular roles of members of the poly(ADP ribose) polymerase (PARP) enzyme family, beyond their known roles in nuclear DNA damage repair. This research has implicated PARP2 as being involved in biomineralisation including vascular and bone calcification processes.

PARP enzymes work by self-PARylating or causing PARylation of neighbouring proteins. This is a NAD-mediated reaction process. It is hypothesised that PAR binds to calcium ions, and therefore that PAR could act as a nidus to concentrate calcium ions to the levels needed for nucleation (ie the start of biomineralisation). PARP inhibitor drugs work by binding to the PARP catalytic domain in preference to NAD, thereby preventing the PARylation reaction occurring.

A drug that inhibits vascular calcification would ideally be a PARP2 inhibitor and not a PARP1 inhibitor. This is because PARP1 is well-known to assist in nuclear DNA damage repair (DDR) (attaching to eg a single-strand DNA break site, and through the PARylation process, signalling to repair proteins to act). Leaving the DDR untouched would be preferable. Specifically inhibiting PARP2, and not PARP1, is not trivial, as PARP1 and PARP2 have very similar catalytic domains.

PARP enzyme assays measure the concentration of a drug required to inhibit PARP enzymes. Due to concerns regarding the appropriateness of existing 3rd party PARP enzyme assays, Professor Duer has built a proprietary PARP assay measuring a direct effect of the PARylation reaction. An example concern regarding PARP enzyme assays is that some of them use biotinylated-NAD rather than NAD, due to the ease of measuring biotin concentrations. However, biotinylating NAD doubles its molecular mass, surely impacting the competitive kinetics between NAD and the inhibitor drug at the PARP catalytic site.

Through Professor Duer's proprietary PARP enzyme assay we have discovered minocycline as a specific PARP2 inhibitor (PARP2 IC_{50} = 2.8uM; PARP1 IC_{50} = 205uM). Neither the existing, approved PARP inhibitor drugs (eg olaparib/LYNPARZA) nor the other members of the tetracycline class of antibiotics (eg doxycycline) are specific PARP2 inhibitors.

Inhibition of PARP enzyme activity using minocycline has been shown to inhibit microcalcification both *in vitro* and *in vivo* using rat models [30]. Whether the same effect is seen in humans is unknown.

2. OBJECTIVES

2.1 Primary hypothesis

Active microcalcification (as measured by NaF TBR_{max}) will be reduced by minocycline.

2.2 Primary Objective

To investigate whether treatment with minocycline reduces microcalcification activity (as measured by NaF TBR_{max}) in symptomatic carotid atheroma compared to 'standard care.'

2.3 Secondary Objectives

- To measure change in microcalcification activity (as measured by NaF TBR_{max}) in non-culprit carotid atheroma compared to 'standard care.'
- To measure biomechanical stress in symptomatic and non-culprit arteries in relation to NaF TBR_{max}.
- To measure change in macrocalcification (as measured using Agatston scoring) in the aortic arch, and symptomatic and non-culprit carotid arteries in the minocycline-treated group and 'standard care.'
- To measure change in serum poly(ADP-ribose) in the minocycline-treated group and 'standard care' group.

2.4 Intended application of study

This study aims to utilise advances in molecular imaging of atheroma to improve the understanding of how microcalcification in atherosclerosis evolves after plaque rupture, and whether this process is arrested in response to minocycline. The application of these imaging techniques to stroke in this way is a novel approach.

The current clinical management of significant carotid stenosis is based upon an individual already having had a stroke/TIA, the degree of anatomical obstruction, and whether the individual is fit enough to undergo vascular surgery. In the case of the latter, older individuals are frequently unable to undergo surgery due to multiple medical co-morbidities that

preclude anaesthetic or would make an operation too risky, leaving them with medical (pharmacological) therapy only. Carotid endarterectomy carries a complication rate of stroke between 1-10% across centres (Barbetta 2013). Carotid stenting procedures are still currently not routine in practice due to high stroke complication rates despite many large-scale trials. There is therefore increasing interest in developing more intensive ‘medical management’ approaches to symptomatic carotid pathology.

3. STUDY DESIGN

3.1 Summary of Study Design

The study is a longitudinal interventional study that involves radiological assessment of microcalcification in carotid atheroma using NaF-PET/CT, and investigates the effect on microcalcification following a 90-day course of minocycline versus 'standard care.'

Patients will be recruited from either the Stroke Unit or the Stroke/Neurology/Neurovascular outpatient clinics. Participants will have had an ischaemic stroke secondary to significant (greater than 30% stenosis that is felt to be causative) carotid disease within the previous seven days. Participants will undergo NaF-PET/CT and CT angiogram (CTA) of the carotid arteries within fourteen days of their ischaemic stroke. This may either be done whilst they are an in-patient on the Stroke Unit or participants may return to have radiological investigations performed as an out-patient. Imaging will be performed at the PET/CT Department at Cambridge University Hospitals NHS Foundation Trust.

After 90 days, carotid NaF-PET/CT and CTA will be repeated. The patient will also be assessed by a clinically-trained member of the study team to assess for any recurrent events.

It is anticipated that a participant's participation will last approximately 90 days. Appendix A shows the timeline of visits and investigations.

3.2 Primary and Secondary Endpoints/Outcome Measures

3.2.1 Primary endpoint

Change in NaF TBR_{max} in symptomatic carotid atheroma in the minocycline intervention group compared to the 'standard care' group.

3.2.2 Secondary endpoints

- Change in NaF TBR_{max} in non-culprit carotid atheroma in the minocycline intervention group compared to the 'standard care' group.
- Measurement of biomechanical stress in symptomatic and non-culprit arteries in relation to NaF TBR_{max}.
- Macrocalcification (measured using Agatston scoring) in aorta (ascending/arch), and symptomatic and non-culprit carotid arteries.
- Further episodes of TIA/stroke symptoms.
- Charge in serum poly(ADP-ribose) concentrations in the minocycline intervention group compared to the 'standard care' group.

3.3 Study Participants

3.3.1 Overall Description of Study Participants

Participants with an ischaemic stroke with symptomatic carotid stenosis of ≥30% will be recruited from either the Stroke Unit or Stroke/Neurology/Neurovascular outpatient clinics at Cambridge University Hospitals NHS Foundation Trust.

3.3.2 Inclusion Criteria

The participant would be eligible to enter the study if they satisfied all of the following:

- Participant is willing and able to give informed consent for participation in the study.
- Male or Female, aged 50 years or above.
- Participants have had an ischaemic stroke or transient ischaemic attack (TIA) in the last 7 days that has been confirmed to be due to atheroma in the carotid artery.
- Have evidence of carotid territory atheroma of at least 30% in the ipsilateral carotid artery to the infarct that is felt to be causative (using Doppler ultrasound or computed tomography angiography).

3.3.3 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- The participant has a medical history or clinically relevant abnormality identified on the screening medical examination, vital sign measurement, or clinical laboratory examination that is deemed by the principal investigator and/or designee to make the subject ineligible for inclusion,
- The participant has had a haemorrhagic stroke.
- The participant is a woman of childbearing potential.
- The participant is in atrial fibrillation.
- The participant has had a previous adverse reaction to minocycline.
- The participant has a medical condition in which the use of minocycline is cautioned: systemic lupus erythematosus (SLE), liver dysfunction, myasthenia gravis, intracranial hypertension, or lactose intolerance.
- The participant is taking medication where there is a significant risk of interaction with minocycline: anticoagulants, penicillins, isotretinoin.
- Planned carotid revascularisation procedure prior to the 90-day re-imaging.
- The participant has evidence of a complete occlusion of their internal carotid artery on the ipsilateral side to the infarct.
- The participant has limited life expectancy due to another illness or chronic condition making follow-up difficult (e.g. widespread malignancy).
- The participant has existing co-morbid medical conditions that would prevent them lying flat in the scanner (e.g. heart failure).
- The participant has known chronic kidney disease that would preclude contrast use (i.e. excluded if eGFR <30ml/min/1.73m^2 as calculated by the Cockcroft-Gault formula).
- The participant is unable to give informed consent.
- The participant is already participating in two other research studies.

3.4 Study Procedures

During this study, the following radiological investigations will be conducted:

^{18}F-sodium fluoride Positron Emission Tomography/Computed Tomography (NaF

PET/CT): NaF-PET/CT will be performed within two weeks of symptom-onset after the acute neurovascular event, and then repeated after 90 days. This will be performed in the PET/CT Department of Cambridge University Hospitals NHS Foundation Trust. A cannula will be inserted and NaF tracer given 60 minutes prior to the PET scan. The patient will wait within the department between the injection and the PET scan. Upon completion of this scan, the cannula will be removed. Due to the NaF, the participant will continue to be radioactive for up to six hours after the procedure and they will be advised not to come into contact with children or pregnant women. At the end of the procedure, the participant will be free to return home or to the ward. The NaF tracer will be acquired from commercial sources through established commercial links with the PET/CT Department at Cambridge University Hospitals NHS Foundation Trust. L.

Computed Tomography Angiography (CTA) of the carotid arteries: A CTA of the carotid arteries will be performed at the same time as the NaF-PET/CT and will require contrast to be given through the cannula at the same time as the CT component of the scan.

Blood sampling for poly(ADP-ribose): Serum blood samples will be taken at the time of cannula insertion to test for serum poly(ADP-ribose) concentrations.

3.5 Study Assessments

Potential participants will be identified as meeting eligibility criteria by the direct healthcare team at the time of admission or clinic review (both within 7 days of an acute ischaemic stroke). Following approach and consent by the potential participant, research team personnel will review the eligibility criteria. At that time, baseline characteristics (age, sex, ethnicity, body mass index (BMI), smoking history, presence of vascular risk factors) will be collected.

3.6 Informed Consent

Potential participants will be identified by the direct healthcare team as fulfilling the required eligibility requirements and will seek their permission for approach by the research team. Members of the research team (medically-qualified staff or stroke specialist research nurses) will then approach the potential participant to discuss the study and answer any questions that they may have. Patient information will be provided at this time. The potential participant will

then be given a period of time (normally not less than twenty-four hours) before a repeat visit by the research team where any remaining questions will be answered and written consent completed.

3.7 Definition of End of Study

The end of study will be the date of the last visit of the last participant.

4. INTERVENTIONS

4.1 Treatment according to Cohort

The treatment regime for the two cohorts in this study will be:

'Standard care'	Aspirin 75mg daily
	Clopidogrel 75mg daily
	Atorvastatin 40mg daily
Intervention	Aspirin 75mg daily
	Clopidogrel 75mg daily
	Atorvastatin 40mg daily
	Minocycline 100mg twice daily

There is no placebo in the study and participants will be aware of their allocation.

Participants will be asked to bring the medication blister packs with them at the final visit of the study to assess concordance with the minocycline in the intervention group. Concordance will also be assessed at the mid-point (six week) consultation (either over the telephone or in person).

4.2 Randomisation to Cohort

Individuals will be assigned in a 1:1 ratio to either the intervention group or standard care. Randomisation will be performed using a sealed envelope randomisation method.

5. ANALYSIS AND STATISTICS

5.1 Analysis of Endpoints

For NaF-PET/CT, co-registered images will be resampled to 3 mm slice thickness and regions of interest (ROIs) were will be manually on fused PET/CT images along the common carotid and internal carotid artery to encompass the region approximately 1 cm proximal and 3 cm distal to the carotid bifurcation (i.e. 3 slices below and 10 slices above the carotid bifurcation, producing 14 slices in total including the carotid bifurcation), as per previously established methodology [31-33]. ROIs will then transferred onto the co-registered PET to produce standardised uptake values (SUV).

SUV represents the ratio of radiotracer concentration in the target tissue to the injected radiotracer activity adjusted for weight. It is given by the equation:

$$SUV = \frac{\text{Tissue radiotracer concentration (MBq/kg)}}{\text{Injected radiotracer activity (MBq) / body weight (kg)}}$$

SUV will be analysed as SUV_{max} and SUV_{mean}. The SUV_{max} is calculated using the highest tissue radiotracer concentration in the ROI whilst SUV_{mean} is calculated using the mean tissue radiotracer concentration throughout the ROI.

Whilst SUV may be used for all types of tissue and ROIs, tissue-to-background ratio (TBR) was developed to correct for blood uptake of radiotracer ("blood pooling"). To calculate TBR readings (TBR_{max} and TBR_{mean}), corresponding carotid SUVs will be divided by venous SUV_{mean}. Venous SUV_{mean} will be calculated by drawing mid-luminal ROIs in the jugular vein over five contiguous 3 mm slices without evidence of spill-over from neighbouring structures, and calculating the mean of the SUV_{mean} of these ROIs [34].

The TBR_{max} and TBR_{mean} for symptomatic and non-culprit carotid arteries will be compared for the single hottest slice (SHS), most diseased segment (MDS), and median whole vessel (WV). The SHS is defined as the single ROI with the highest tracer uptake for the artery. The MDS considers the most diseased 6 mm of the artery (based on tracer uptake) and

represents the mean of ROIs of three contiguous axial slices where the central ROI is the SHS, along with the slice immediately proximal and immediately distal to SHS, as per previous methodology [35]. Finally, the WV is the median of tracer uptake in all 14 axial slices of the artery (see **Figure 5**).

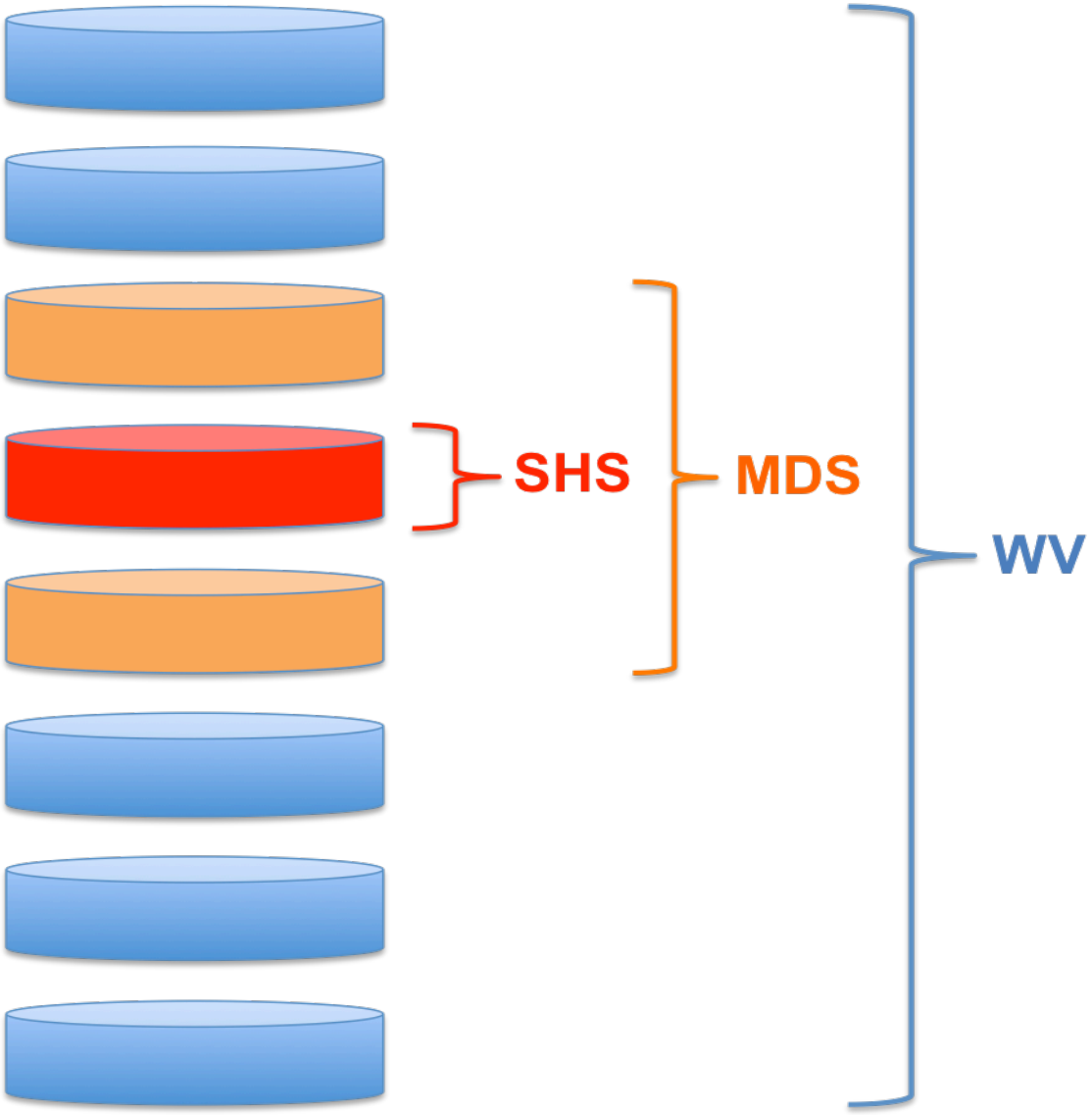


Figure 5: Schema of the relationship between SHS, MDS, and WV readings. The single hottest slice (SHS) is the axial slice with the highest radiotracer uptake; the most diseased segment (MDS) is the mean of the three contiguous axial slices surrounding the SHS; whole vessel (WV) is the median of all 14 axial slices along the carotid artery.

5.2 The Number of Participants

Based on rat models, there was an approximate 50% reduction in vascular tissue microcalcification after treatment with minocycline [30]. In the ICARUSS study, the MDS TBR_{max} in culprit arteries was 2.85 (SD 1.15), and in non-culprit was 2.34 (SD 0.64) [36]. Hence, a sample size of 12 in each group (alpha 0.05, beta 0.2) would allow us to detect a 50% reduction in symptomatic plaque and/or 33% reduction in non-culprit plaque.

In the instance of non-completion of the study, we will replace the study participant with another participant allocated to the same cohort. In the current MINERVA study (evaluating the use of minocycline in neuroinflammation) there have been no reported side-effects in those taking minocycline as the study drug, and we anticipate a similar low level of intolerance in the MINOTAUR study.

5.3 Interim Analysis

An interim analysis will be conducted once the first six in each cohort have completed study participation.

6. SAFETY REPORTING

6.1 Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

6.2 Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was: ‘related’ – that is, it resulted from administration of any of the research procedures; and ‘unexpected’ – that is, the type of event is not listed in the protocol as an expected occurrence. Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES report of serious adverse event form (see NRES website**).**

7. ETHICS

7.1 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a randomly-generated participant ID number on any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act that requires data to be anonymised as soon as it is practical to do so.

7.2 Radiation exposure

Addition radiation exposure of the study is as follows:

125 MBq NaF x 2	2.2 x 2 = 4.4 mSv
CT for attenuation correction	0.4 x 2 = 0.8 mSv
CT angiogram x 2	3.4 x 2 = 6.8 mSv
Total	**12 mSv**

For comparison, the dose to participants is equivalent to less than 5 years of exposure to natural background radiation in the UK. Using a risk estimate of detriment of 4.2 % / Sv from ICRP 103, the estimated risk of cancer (fatal or non-fatal) and of severe hereditary risks for the total research protocol dose is about 1 in 2000. This can be considered in the light of the natural incidence of fatal cancer, which is of the order of 1 in 4.

7.3 Incidental findings

During the study, incidental findings (such as unexpected radiological findings, significant worsening of cerebrovascular disease or evidence of significant untreated depression) may be discovered. In such cases, the participant will be reviewed in the stroke out-patient clinic and the findings discussed with them. Depending upon the finding, the participant's consent may be sought to refer them to another hospital specialist or to their General Practitioner for further evaluation. This procedure is also detailed in the participant information literature given to potential participants prior to enrolment in the study.

7.4 Loss of capacity during the study

If a participant loses capacity to consent to ongoing participation in the study then they will be withdrawn from the study. No further data or imaging will be collected/conducted, though data collected prior to withdrawal may still be used. If allocated to the minocycline intervention, then this will be discontinued. Such withdrawals will be replaced as per section 5.2.

8. DATA HANDLING AND RECORD KEEPING

Study participants will be allocated a randomised study participant number at the time of enrolment. Radiological images will be anonymised at the time of scan with this anonymised participant number and will be stored electronically on a secure University of Cambridge server hosted by the Wolfson Brain Imaging Centre (WBIC). Images will be analysed and data generated using OsiriX imaging software.

Paper copies of the CRF will be anonymised with the randomised study participant number and kept securely within locked storage located in the R3 Department of Clinical Neurosciences research offices. Access to the locked storage is limited to study personnel only. Access to the research offices is restricted to research personnel and requires keycode access outside of working hours. An electronic record of imaging and CRF data will be stored in an encrypted Microsoft Excel document stored on the secure University of Cambridge server hosted by the Wolfson Brain Imaging Centre. The name and any other identifying detail will NOT be included in any study data electronic file.

Participant information will be retained for one year after the study. Paper and electronic copies of study data will be kept for 15 years. Imaging data will be kept securely possibly indefinitely in the NHS data archive in accordance with good research practice.

In line with good research practice, anonymised data may be used by researchers working within the team for other similar ethically approved research studies, where the same standards of confidentiality will apply. Anonymous data may also be disclosed to researchers outside of the host institution when such individuals are working in close collaboration with the research team. Sharing of such data will be at the discretion of the Principal Investigator and would require the recipient to sign a Code of Conduct guaranteeing that the data will be kept confidential and securely. Requests for sharing of data will be subject to periodic review by an independent Access Advisor.

9. FINANCING AND INSURANCE

Funding for the MINOTAUR study has been provided by Varsity Pharmaceuticals. Varsity Pharmaceuticals has had no role in the design of the protocol, and will have no role in the analysis of data or interpretation of the results.

The University Insurance Manager has advised that insurance for negligent and non-negligent harm under the University's Clinical Trials policy can be arranged if this trial is approved by the NHS ethics committee. The University's insurers are Newline, the insurance policy reference is B0823Q31000177 and the Limit of Indemnity under this policy is £10m.

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the clinical trial caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the trial, but no-one has acted negligently.

The University of Cambridge will arrange insurance for negligent harm caused as a result of protocol design and for non-negligent harm arising through participation in the clinical trial.

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APPENDIX A: TIMELINE OF PARTICIPATION.

Visit 1 (within 14 days of stroke)	Combined NaF-PET/CT and CTA. Serum blood sampling for poly(ADP-ribose)
Visit 2 (after 6 weeks)	Clinical review (either over the telephone or in person as required) Dispensing of minocycline (in minocycline arm)
Visit 3 (at 90 days)	Repeat combined NaF-PET/CT and CTA. Serum blood sampling for poly(ADP-ribose)