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A study protocol for SFX-01 After Subarachnoid haemorrhage (SAS): A multi-centre randomised double-blinded, placebo controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-028514
Article Type:	Protocol
Date Submitted by the Author:	13-Dec-2018
Complete List of Authors:	Zolnourian, Ardan; University of Southampton, Clinical Neuroscience; University Hospital Southampton NHS Foundation Trust, Galea, Ian; University of Southampton Faculty of Medicine, Experimental Neurology Bulters, Diederik ; University Hospital Southampton NHS Foundation Trust
Keywords:	Randomised controlled trial, Subarachnoid haemorrhage, Nrf2, Sulforaphane, Delayed cerebral ischaemia

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3 **A study protocol for SFX-01 After Subarachnoid haemorrhage**
4 **(SAS): A multi-centre randomised double-blinded, placebo**
5 **controlled trial**
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10 **Date and version:** 16th May 2018, Version 7

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Abstract

Introduction

Subarachnoid haemorrhage from a ruptured cerebral aneurysm carries a high morbidity and mortality with subsequent negative impact on society and healthcare. Despite huge advances in techniques to secure the aneurysms, there has been little progress in the treatment of the deleterious effects of the haemorrhage.

Sulforaphane is an Nrf2 inducer with anti-oxidant and anti-inflammatory properties. It has been shown to improve clinical outcome in experimental models of SAH, but it is unstable. SFX-01 (Evgen Pharma) is a novel composition comprised of synthetic sulforaphane stabilised with α -cyclodextrin complex. On ingestion, the complex releases sulforaphane making SFX-01 an ideal vehicle for delivery of sulforaphane.

Methods and analysis

The objective of the study is to assess the safety, pharmacokinetics and efficacy of SFX-01. This is a prospective, multi-centre, randomised, double-blind placebo-controlled trial in patients aged 18-80 years with subarachnoid haemorrhage in the previous 48 hours. They will be randomised to receive SFX-01 or placebo twice daily for up to 28 days.

Pharmacokinetics will be assessed based on paired blood and CSF sulforaphane and its metabolites levels on day seven and in a subgroup of patients on hourly samples taken during six hours post-dosing on days one and seven. Pharmacodynamics will be assessed by haptoglobin and malondialdehyde levels, and maximum flow velocity of middle cerebral artery will be measured by transcranial Doppler ultrasound.

Clinical outcomes will be assessed at days 28, 90 and 180 using combination of mRS, GOSE, SAHOT, SF-36, BICRO-39 and CLCE-24. Further secondary outcomes include MRI at six months; quantitative susceptibility mapping will be used to measure iron deposition and volumetric T1 images for cortical volume.

Ethics and dissemination

Appropriate ethical approval was obtained from the relevant research ethics committee. All the results and outcomes of the trial will be outlined and submitted for publication in a peer reviewed journal.

Trial registration number: 2014-003284-38 ; Pre-results

Strengths and limitations of this study

- **SFX-01 is a novel complex of sulforaphane and cyclodextrin that can deliver high levels of sulforaphane in the clinical environment, offering the opportunity for the first time to reproduce the positive results of sulforaphane treatment in experimental models of subarachnoid haemorrhage.**
- **The trial is at low risk of bias due to placebo control and appropriate blinding.**
- **In addition to clear primary outcomes there are a wide range of secondary outcomes to probe the mechanisms underlying any efficacy of the treatment including novel and new outcome measures and MRI sequences.**
- **Although it is a multicentre trial, due to the complexity of its design, it has been limited to three centres to ensure high quality outcome measurement.**

Introduction

Spontaneous Subarachnoid Haemorrhage (SAH) is a devastating cerebrovascular injury with an incidence of 9.1 per 100,000 population [1]. It affects around 7000 patients in the UK annually. Around 85% are due to ruptured intracranial aneurysms [2]. The incidence is age related peaking at 52 years. SAH carries a high overall mortality rate of up to 67% [3], and only half of the survivors are able to live independently [4]. It therefore has a high burden on society due to the loss of productivity and resources [5].

Conventionally following SAH, treatment is primarily directed to securing the aneurysm to prevent further re-bleeding. This however does nothing to ameliorate the morbidity and mortality due to the haemorrhage. The only approved treatment is nimodipine [6]. However, its effects are small and poor outcome remains a significant problem [7]. Moreover, even in survivors considered to have made a good recovery, neurocognitive deficits are common leading to extensive problems with social reintegration and functioning in the workplace [5].

The mechanism underlying poor outcome is multifactorial. A significant component is due to secondary injury from oxidative stress, inflammation [8], spreading depolarisation [9], macroscopic cerebral vasospasm [10] and microcirculatory disturbance [11]. The common factor is that they are initiated by extracellular haemoglobin (Hb) released as red blood cells in the clot lyse. This results in direct neurotoxicity, increased oxidative stress and further injury [12].

Sulforaphane (SFN) is known to upregulate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. Nrf2 is a redox-sensitive transcription factor that binds to a specific DNA site, the anti-oxidant response element, upstream of genes encoding detoxifying and anti-oxidant enzymes [13] [14]. Some of these enzymes include glutathione S-transferases (GSTs), NAD(P)H-quinone oxidoreductase 1 (NQO1) and haem oxygenase 1 (HO-1) [13]. During physiological conditions Nrf2 is bound to the Kelch-like ECH associated protein 1 (KEAP1) in the cytoplasm. In response to stress such as SAH, Nrf2 is released from KEAP1 and then translocates to the nucleus leading to enhanced gene transcription [15].

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4 Nrf2 also upregulates haptoglobin (Hp), an acute phase glycoprotein found in plasma
5 [16], as well as cerebrospinal fluid (CSF) [17]. Hp is part of an important Hb
6 scavenging pathway after SAH. It tightly binds to free Hb, and this Hb-Hp complex is
7 taken up by CD163-positive macrophages [18]. This pathway is saturated after SAH
8 [17] and upregulation of Hp represents a possible therapeutic avenue [19]. Nrf2 also
9 regulates degradation of red blood cells, and metabolism of haem and iron through
10 transcriptional upregulation of CD36 [20] [20], haemopexin [21], HO-1 [22] and
11 ferritin [23].

12
13 Nrf2 is expressed in the central nervous system (CNS) and is upregulated in response
14 to inflammation and cerebral insults [24]. Nrf2 knockout is associated with a more
15 pronounced inflammatory response *in vitro* [25] and *in vivo* [26], and the increased
16 inflammatory response is associated with more brain oedema, cell death and poorer
17 neurological recovery [27]. SFN increases HO-1, NQO1, and GST- α 1 levels and
18 reduces IL-1 β , IL-6, and TNF- α [28]. It leads to a reduction in vasospasm and
19 improves neurological recovery [26] [29].
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22
23 SFN has a relatively short half-life rendering it impractical for clinical use. SFX-01
24 (Evgen Pharma) is a novel new agent comprising SFN complexed with cyclodextrin
25 which is suitable for clinical use. On ingestion, SFN is released from the cyclodextrin
26 and is an effective method to deliver SFN. In two Phase I trials (NCT01948362,
27 NCT02055716) no serious adverse events were reported in healthy volunteers. Here
28 we describe the protocol for a phase II trial of SFX-01 in patients who have suffered a
29 SAH.
30

31 **Objectives**

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33 The objective of the study is to assess the safety, pharmacokinetics and efficacy of
34 SFX-01.
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37 **Methods**

38 ***Trial design***

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40 This is a prospective, double-blind, parallel group, randomised controlled trial
41 comparing SFX-01 (300 mg) taken orally as capsules or as a suspension via a
42 nasogastric tube (NG) twice-daily for up to 28 days *versus* placebo in patients with
43 SAH within 48 hours of enrolment.
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49 The treatment window was selected based on clinical and biochemical criteria. After
50 SAH red blood cells in the subarachnoid space lyse and gradually release Hb. This
51 lysis takes time and initially any free haemoglobin is bound by Hp. Only with
52 progressive Hb accumulation do levels of free Hb rise with the most marked increase
53 occurring after three days [30]. Clinically delayed cerebral ischaemia (DCI) is only
54 seen after three days. Therefore, initiation of treatment should ideally occur within the
55 first 72 hours, to allow more time for Nrf2 pathway activation and expression of its
56 transcriptome. However not all patients present and/or are diagnosed immediately.
57 Since a local audit at the main study neurosurgical centre showed that most patients
58 are admitted within 48 hours, initiation of treatment within 48 hours was established
59
60

as an inclusion criterion, striking the best compromise between the practicality of study recruitment and the need to start treatment early.

Detailed pharmacovigilance will inform the safety of SFX-01. Pharmacokinetics will be determined by measurement levels of SFN and its metabolites in CSF and blood at day seven in all patients, and a more detailed profile will be obtained in a subgroup of 12 patients who will have hourly blood and CSF samples for six hours after dosing on days one and seven. Pharmacodynamics will be assessed with blood Hp and malondialdehyde (MDA) levels, and middle cerebral artery (MCA) flow velocity as measured on transcranial Doppler (TCD) ultrasound as an estimate of large cerebral artery spasm. Longer term outcome will be assessed using validated outcome scales, at day 28 using Modified Rankin Scale (mRS) ([31] [32] [33]), Glasgow Outcome Score (GOSE) [34][35] and the SAH Outcome Tool (SAHOT) [36] as well as at day 90 and 180 using the mRS, GOSE, SAHOT, Brain Injury Community Rehabilitation Outcome Scales (BICRO-39) [37] and the Check List for Cognitive and Emotional consequences following stroke (CLCE-24) [38].

Patient and public involvement

The study was developed with the assistance of the Wessex Subarachnoid Haemorrhage Support Group. The research team met with members on several occasions for research priority setting, trial design, and development of outcome scales. At an early priority setting session the long-term symptoms from the haemorrhage were identified as the main priority for research and medical management preferred over surgical interventions. As a result, a proposal for a trial was made which was subsequently discussed to obtain views on the individual study interventions and how to minimise inconvenience to patients. Options for consent were also discussed and patients helped develop this process and contributed to our patient information sheets. Over two separate meetings the main symptoms after SAH were identified and developed into SAHOT, the first SAH specific outcome scale, utilised in this study. Upon completion of the trial participants will be informed of the results which will also be distributed through the Wessex Subarachnoid Haemorrhage Support Group.

Study setting

This is a multicentre study conducted in regional neurosciences centres with specialist services to treat aneurysmal SAH. Patients will be identified at referral to the admitting neurosurgical or neurointensive care units. After informed consent, dosing and study interventions will occur in the neurosciences centre until patients are discharged to either home or their local hospitals. There are three recruiting centres; University Hospital Southampton, Royal Infirmary of Edinburgh and the Royal London Hospital. Upon discharge, follow-up may take place in clinic, or at visits to district general hospitals and rehabilitation centres, or patients' residences.

Eligibility Criteria

Inclusion criteria

1. Patients with radiological evidence of spontaneous aneurysmal SAH

2. Fisher grade 3 or 4 on CT
3. Definitive treatment of aneurysm has not been ruled out
4. Previously living independently
5. In the opinion of the investigator, the delay from ictus to randomisation and initiation of trial medication will not exceed 48 hours
6. Aged 18 to 80 years
7. In the opinion of the investigator it will be possible to obtain Informed Consent from the Patient, Personal Legal Representative or Professional Legal representative within 24 hours of first dose

Exclusion criteria

1. Traumatic SAH
2. Fisher grade 1 or 2
3. SAH diagnosed on Lumbar puncture (LP) with no evidence of blood on CT
4. Decision not to treat aneurysm has been made
5. Plan to withdraw treatment
6. Significant kidney disease as defined as plasma creatinine ≥ 2.5 mg/dL (221 μ mol/l)
7. Liver disease as defined as total bilirubin ≥ 2 -fold the upper limit of normal, as measured by the local laboratory
8. Females who are pregnant or lactating
9. Participants enrolled in another interventional research trial in the last 30 days
10. Patients for whom it is known, at the time of screening, that clinical follow-up will not be feasible
11. Patients unwilling to use two forms of contraception (one of which being a barrier method) for 90 days (men) or 30 days (women) after the last trial medication dose
12. Known hypersensitivity to any component of a SFN containing product including broccoli

Recruitment is limited to Fisher grade 3 and 4 SAH. These patients represent the majority of aneurysmal SAH. They have a higher volume of haemorrhage with a poorer outcome and more delayed neurological deficits [39]. They are therefore mechanistically and clinically expected to derive greatest benefit from SFX-01.

Inclusion of patients with unsecured aneurysms would risk a high incidence of rebleeding in the study. Rebleeding is associated with exceedingly bad outcomes [40], masking any effect from SFX-01. However, since not all aneurysms are secured within 48 hours, there is no requirement for the aneurysm to have been secured prior to enrolment. Instead patients in whom treatment of the aneurysm has been ruled out due to poor clinical status will be excluded.

Although there are no known risks to kidney or liver, due to the relative inexperience with SFX-01 in humans, patients with liver or kidney problems are excluded.

Intervention

Trial medication

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3 SFX-01 (active 300 mg capsule) or placebo (cyclodextrin only capsule) will be taken
4 orally or as a suspension via a nasogastric tube (NG) twice daily for up to 28 days.
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7 Animal studies in ischaemic stroke, intracerebral haemorrhage and SAH have all used
8 5 mg/kg dose of SFN in rodents [26] [41] [42] [43]. Conversion of animal doses to
9 humans using body surface area, as has been widely recommended [44] [45], yields a
10 human dose of 50 mg SFN. This is equivalent to 300 mg of SFX-01 containing 46.15
11 mg of SFN. In the clinical studies conducted to date, SFX-01 has been shown to be
12 well tolerated at doses of 600mg once daily and 300mg twice daily with no serious
13 adverse effects.
14

15 16 ***De-escalation from the trial regimen***

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18 In the event of tolerability problems whilst the patient is in the neurosurgical centre,
19 the Investigator will assess whether simple measures to ease the effects of the adverse
20 event(s) may be implemented (i.e. antacid in the case of GI irritation or anti-emetic in
21 the event of nausea).
22

23 The investigator will also assess whether or not the adverse event(s) could be related
24 to the trial medication and severe enough to warrant a dose frequency reduction. In
25 the first instance the investigator may consider missing one dose. If a dose frequency
26 reduction is warranted, from that point onwards the second dose of the day will be
27 omitted; a dose frequency increase back to twice daily will not be permitted.
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30 If tolerability problems continue then the investigational medication will be stopped;
31 patients will continue in the study and complete the study visits. The staged dose
32 frequency de-escalation (dropping to once daily) will not be carried out after
33 discharge from the neurosurgical centre; if tolerability problems occur after discharge,
34 medication will be stopped; patients will continue in the study and complete the study
35 visits in accordance with the schedule of assessments.
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37 38 ***Treatment compliance***

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40 Compliance with treatment will be recorded during the inpatient hospital stay by
41 health care professionals and/or a member of the research team. On discharge to the
42 usual residence, responsibility for this is transferred to the patient or their Personal
43 Legal Representative, aided by detailed instructions. In the event of discharge to a
44 rehabilitation unit or patient local hospital, written instructions will be provided on
45 discharge and verbal communication with the clinical team will ensure compliance.
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48 All patients will be discharged with a patient diary which will be filled in and
49 collected at day 28. Compliance will be further monitored by drug reconciliation.
50 Patients will be asked to return the medication bottle and any residual contents at the
51 day 28 visit. At this time any residual tablets will be counted and recorded.
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54 55 ***Concomitant treatment***

56 There are no known drug interactions, and participation in the trial will not alter
57 routine treatment of SAH. Participation in other interventional research studies will
58 not be allowed until after the last follow up visit.
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Outcomes

Primary end-points

Safety

To evaluate the safety of up to 28 days of SFX-01 dosed at up to an equivalent of 92mg SFN per day.

Prior to the start of this study, the trial medication had only been used in healthy individuals and not in a patient population. Therefore safety is one of the main objectives of this study. This is evaluated through routine tests (full blood count, urea and electrolytes, coagulation screen, liver function tests, and urine microscopy) at baseline, post-dose, day seven and day 28 as well as close monitoring of patients for any side-effects or adverse events.

Pharmacokinetic

To detect the presence of SFN and its metabolites in CSF and blood.

Animal models have shown that SFN crosses the blood-brain barrier and can therefore be detected in the brain [46] [47] There is some variation in the levels achieved in these studies, and it has not been studied in humans. All patients will have a paired CSF/blood sample taken at seven days post-ictus. This will be via a LP unless the patient has an external ventricular drain (EVD) for their clinical care in which case it will be obtained from the EVD. In addition, up to 12 patients with an EVD will be asked to consent to hourly CSF samples for six hours after dosing on days one and seven.

Vasospasm

To determine if a minimum of seven days treatment with SFX-01 reduces MCA peak flow velocity following SAH.

TCD ultrasound will be used to measure the MCA, ICA and ECA maximum flow velocity and the Lindegaard ratio will be calculated. TCDs will be performed on alternate daily basis including at baseline. They will be performed for at least seven days or until no longer clinically indicated. Blood flow velocity is measured in cm/second and is inversely related to the luminal diameter of the vessel. The greater this value the more likely the degree of narrowing or spasm in the vessel. It has a good predictive value for DCI. A recent systematic review and meta-analysis, which pooled data from 2870 patients, showed a sensitivity of 90%, specificity of 71%, and negative and positive predictive values of 92% and 57% respectively [48].

Secondary end-points

Pharmacodynamic

CSF and blood – Haptoglobin and Malondialdehyde

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4 Hp represents a major Hb detoxification pathway and is upregulated by Nrf2. MDA is
5 a measure of oxidative stress. Hp and MDA will be measured in blood at baseline,
6 day seven and day 28 and in CSF on day seven. A local audit at the main study
7 neurosurgical centre showed that approximately 1/3 of patients have an EVD sited as
8 part of their routine clinical care to treat hydrocephalus. In these patients additional
9 samples will be obtained. This will allow investigation of the temporal profile of Hp
10 level and oxidative stress. Additionally, there will be exploratory investigations using
11 proteomics, transcriptomics and genomics using CSF and blood samples.
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14 ***MRI – iron and brain volume***

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17 All patients will have magnetic resonance imaging (MRI) 180 days after SAH. Brain
18 volume on T1 sequences will be measured, since this has been shown to correlate
19 with outcome [49]. Cortical iron content will be assessed using quantitative
20 susceptibility mapping after susceptibility weighted MR imaging, which
21 predominantly measures siderotic iron deposits [50]. Iron is a major component of
22 Hb, and it is unknown what effects SFX-01, SFN or increased Hp binding of Hb may
23 have on the downstream iron pathway.
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26 ***Clinical outcome***

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- mRS at seven days, discharge, 28, 90 and 180 days.
 - Incidence of DCI defined as a new focal deficit or reduction in GCS (by two points or more) if not explained by other causes (i.e. re-bleed, hydrocephalus, seizure, meningitis, sepsis or hyponatraemia) [51]
 - Incidence of new cerebral infarct on CT or MRI
 - Institution of hypertensive therapy for presumed DCI
 - SF-36 quality of life survey at 28, 90 & 180 days
 - CLCE-24 and BICRO-39 at 90 & 180 days
 - SAHOT and GOSE at 28, 90 & 180 days
 - Length of acute hospital stay
 - Discharge destination

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A number of measures of efficacy have been selected reflecting the most common stroke outcome assessment (mRS), the most common brain injury assessment (GOSE), the most common quality of life survey (SF-36) and the only SAH specific outcome tool [36] to determine the most sensitive tool and make estimates of effect size. SAHOT includes 56 items dealing with cognitive, physical, and behavioural/psychological consequences of SAH, developed in a SAH focus group by patients and experts in the field. This outcome tool has been validated and proposed as a more sensitive and responsive tool in SAH [36].

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A number of short term patient outcomes related to the incidence of DCI are included (incidence of infarction, and institution of hypertensive therapy). DCI is a serious complication following SAH and responsible for significant secondary injury [10]. Several simple outcomes including length of stay and discharge destination that have been associated with outcome [52] are also included in the event any patients are lost to longer term follow up.

Schedule of assessments

Time points ¹	D 0-2	D 1-3 (12-24 post- dose)	Ongoing assessment (Alternate days) ⁶ (+/-1)	D 7 (+/-1)	Discharge (-2)	D 28 (-6/+2)	D 90 (+/-14)	D 180 (+/- 28)
Study procedures								
Consent	X							
Inclusion/exclusion	X							
WFNS grading	X							
IMP treatment	X							
Safety bloods ²	X	X	X	X	X	X		
Safety urine	X			X	X	X		
Lipid profile	X			X	X	X		
Coagulation screen								
TCD readings ³		X	X	X				
HP, MDA (Blood/CSF) ⁴	X			X		X		
SFN & metabolites (Blood/CSF) ⁵				X				
mRS				X	X	X	X	X
GOSE						X	X	X
SAHOT						X	X	X
SF-36						X	X	X
BICRO-39							X	X
CLCE-24							X	X
MRI								X

- 1- Ictus is defined as the onset of symptoms/haemorrhage and is referred to as day 0.
- 2- Safety bloods include: Biochemistry: Sodium, Potassium, Urea, Creatinine, Glucose, Calcium, Total Bilirubin, Alkaline Phosphatase, Alanine Transaminase, Albumin, C-Reactive Protein, and Haematology including Haemoglobin, White Blood Cell Count, Neutrophils (Absolute), Lymphocytes (Absolute), Platelets. These will be done at least on alternate days until no longer clinically indicated.
- 3- TCDs are performed at baseline before day 3 and will be repeated on alternate daily basis until at least day 7 or where clinically indicated.
- 4- HP and MDA will be assayed in both CSF and blood at baseline, where possible i.e if patient has an EVD fitted this will be measured in the CSF and blood at baseline as well as every other day until EVD is removed. All patients will have HP and MDA assayed on either a LP or EVD sample on day 7.
- 5- SFN and its metabolites will be measured on day 7 in all patient with paired blood and CSF (LP or EVD sample).
- 6- These assessments will be done on every other day basis with a +/-1 window. They will be carried on until discharge or up to when it is clinically required.

Sample Size

No formal sample size calculation has been carried out; the power associated with a sample size of 90 is based on the following assumptions:

- The error probability for the Type I error should not exceed 5% for a one-sided test;
- The primary endpoint will be compared between treatment groups by means of a t-test
- The mean maximum MCA flow velocity for patients treated with SFX-01 is estimated as 175 cm/s and

- The standard deviation of maximum MCA flow velocity is 50 cm/s

Under these assumptions 90 patients will give 80% power to detect a difference in maximum MCA velocity which is approximately half of the standard deviation of the mean value. The standard deviation was assumed to be approximately 30% of the mean value.

Recruitment

Patients with SAH who present to the clinical centres and meet the above criteria will be considered for recruitment. The inclusion and exclusion criteria reflect national practice. Patients will be identified by the treating clinical team at the time of referral, admission or daily medical handover.

Assignment of intervention

Randomisation and blinding

Patients will be randomised in a 1:1 ratio to the active or placebo arm. Randomisation is stratified using the most recent World Federation of Neurosurgical Societies (WFNS) grade [53] prior to randomization. Patients in different WFNS groups will have significantly different outcomes [54] and imbalance between treatment arms risks treatment allocation bias.

All treatment packs will be otherwise identical in appearance. Placebo capsules will be identical and contain cyclodextrin making the contents indistinguishable should they be opened either inadvertently or for the purposes of NG administration. Patients will be randomised to one of the treatment groups by allocation of the appropriate, sequentially numbered treatment pack. The treatment packs will be pre-numbered according to a block balanced randomisation code with a ratio of 1:1 by a blinded third party. They will be selected as per WFNS grading by a member of a research team from pharmacy.

Unblinding

The Pharmacy will receive a sealed envelope containing the identity of each trial medication bottle. An envelope may be opened only in the case of a serious adverse event and only when it is essential to the subsequent management of the patient. The independent trial centre will be responsible for breaking codes for regulatory submissions of Suspected Unexpected Serious Adverse Reactions (SUSARs), thereby maintaining the overall confidentiality of the code breaks. If the code is broken the data for that patient will be excluded from the Per Protocol Population analysis but included in the Intention to Treat Analysis. They will continue in the study and complete the study visits in accordance with the study visit schedule.

Data collection and management

Data collection will be performed by Good Clinical Practice-trained members of the research team. Study specific training and additional training in disease specific

questionnaires will be provided. The data will be entered into a secure electronic case report form.

Statistical analysis

The following populations will be considered for the analysis:

- Intention-to-Treat population (ITT): all randomised patients who receive at least one dose of study medication and with any post-dose efficacy evaluations. Patients where the time from ictus to admission is unknown are to be considered as part of the ITT population
- Per-protocol population (PPP): The Per Protocol Population (for Primary analysis) will be considered to be those patients in the ITT population that have been dosed for a minimum to day seven post ictus without any major protocol violations (i.e. wrong inclusions, etc.).
- Safety population: all randomised patients who have taken at least one dose of study medication.

The final full statistical analysis plan will be published prior to unblinding.

Monitoring

A data safety monitoring board (DSMB) has been set-up to monitor safety aspect of the trial throughout. A steering committee (comprising the Chief Investigator and the sponsor's Chief Medical Officer) will receive and review the reports from DSMB, and take action as appropriate. The DSMB plan to hold an initial meeting after recruitment of 20 patients who will have completed seven days of trial medication and if there are no safety concerns, will allow patients to be dosed for up to 28 days and allow patients to be discharged to other hospitals or home with the trial drug. The DSMB will also meet if there are any SUSARs or if two patients have had an increase in the grading of the severity of adverse events.

Recruitment will stop once the target has been reached or if DSMB will deem the study or trial drug to be associated with a significant number of adverse events compared to the normal patient population. The recruitment target is set to a minimum of 90 patients between three centres in the United Kingdom. Up to 120 patients may be recruited in order to allow for withdrawals and deaths.

Adverse events reporting

Adverse events, adverse drug reactions and serious adverse events (SAE) will be accurately documented. SAEs will be reported within 24 hours of awareness. Pregnancies occurring during the study must be reported immediately and monitored closely.

Ethical considerations and Informed Consent

Consent procedures and emergency dosing

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3 Most patients with acute SAH present with either severe headache or altered level of
4 consciousness. Many will lack capacity with no legal representative immediately
5 available. SAH is an acute emergency and any benefit from SFX-01 is likely to be
6 greatest the earlier it is administered. The study has therefore been granted ethical
7 permission to obtain baseline blood testing and administer two doses of trial drug
8 without consent if the patient is lacking in capacity and no legal representative is
9 available. If no consent can be obtained at that point, the patient will be withdrawn
10 from the study.
11
12

13 Consent will be obtained in one of three scenarios:

- 14 1- Patients with capacity
- 15 2- Patients without capacity, but with a relative or next of kin available
16 immediately in person
- 17 3- Patients without capacity and no relative or next of kin immediately available
18 in person. Professional legal representative will be approached.
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23 ***Re-Consent***

24 This must take place in two different scenarios:

- 25 1- Where patients regain capacity they must be re-consented.
- 26 2- Where patients have been consented through a professional legal
27 representative, after which time either patient has regained capacity or the next
28 of kin has become available.
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32 **Discussion**

33 There is mounting evidence for the role of the Nrf2 pathway in outcome after SAH.
34 SFN upregulates Nrf2 expression and improves outcome in animal models. SFX-01
35 represents an exciting and novel way to deliver SFN to patients SAH with the
36 potential to improve their lives.
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40 This trial will investigate the safety, pharmacokinetics and pharmacodynamics of
41 SFX-01 after SAH. If successful it may deliver long-term benefit to patients who have
42 suffered SAH and provide new hope to a group of patients characterised by complex
43 neurocognitive problems and disability.
44
45

46 **Footnotes**

47 **Authors' contributions:** DB conceived the trial. AZ, IG and DB were all involved in
48 the design of the study and its setup. AZ and DB wrote the study protocol. DB
49 managed the recruitment of other centres. IG and DB reviewed the protocol
50 manuscript and approved the final version.
51
52

53 **Funding:** This study is sponsored by EvgenPharma.

54 **Competing interests:** None declared.
55
56

57 **Ethics approval:** South central Research Ethics Committee, Hampshire A, UK.

58 **Word count:** 4192
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References

- 1 de Rooij NK, Linn FHH, van der Plas JA, *et al.* Incidence of subarachnoid haemorrhage: a systematic review with emphasis on region, age, gender and time trends. *J Neurol Neurosurg Psychiatry* 2007;**78**:1365–72. doi:10.1136/jnnp.2007.117655
- 2 Van Gijn J, Rinkel GJE. Subarachnoid haemorrhage: Diagnosis, causes and management. *Brain*. 2001. doi:10.1093/brain/124.2.249
- 3 Nieuwkamp DJ, Setz LE, Algra A, *et al.* Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. *Lancet Neurol* 2009;**8**:635–42. doi:10.1016/S1474-4422(09)70126-7
- 4 Kirkpatrick P, Lindsay K, Shaw M, *et al.* *National Study of Subarachnoid Haemorrhage*. 2006.
- 5 Rivero-Arias O, Gray A, Wolstenholme J. Burden of disease and costs of aneurysmal subarachnoid haemorrhage (aSAH) in the United Kingdom. *Cost Eff Resour Alloc* 2010;**8**:1–12. doi:10.1186/1478-7547-8-6
- 6 Pickard JD, Murray GD, Illingworth R, *et al.* Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage : British aneurysm nimodipine trial. *Bmj* 1989;**298**:636–42. doi:10.1136/bmj.298.6674.636
- 7 Dorhout Mees SM, Rinkel GJ, Feigin VL, *et al.* Calcium antagonists for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev* Published Online First: 2007. doi:10.1002/14651858.CD000277.pub3
- 8 Fassbender K, Hodapp B, Rossol S, *et al.* Inflammatory cytokines in subarachnoid haemorrhage : association with abnormal blood flow velocities in basal cerebral arteries Inflammatory cytokines in subarachnoid haemorrhage : association with abnormal blood flow velocities in basal cerebral arteries. *Psychiatry Interpers Biol Process* 2001;:534–7. doi:10.1136/jnnp.70.4.534
- 9 Dreier JP, Major S, Manning A, *et al.* Cortical spreading ischaemia is a novel process involved in ischaemic damage in patients with aneurysmal subarachnoid haemorrhage. *Brain* 2009;**132**:1866–81. doi:10.1093/brain/awp102
- 10 Dorsch NWC, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage Part I: Incidence and effects. *J Clin Neurosci* 1994;**1**:19–26. doi:10.1016/0967-5868(94)90005-1
- 11 Pennings FA, Bouma GJ, Ince C. Direct observation of the human cerebral microcirculation during aneurysm surgery reveals increased arteriolar contractility. *Stroke* 2004;**35**:1284–8. doi:10.1161/01.STR.0000126039.91400.cb

- 12 Gaetani P, Pasqualin A, Rodriguez y Baena R, *et al.* Oxidative stress in the human brain after subarachnoid hemorrhage. *J Neurosurg* 1998;**89**:748–54. doi:10.3171/jns.1998.89.5.0748
- 13 Itoh K, Chiba T, Takahashi S, *et al.* An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 1997;**236**:313–22. doi:10.1006/bbrc.1997.6943
- 14 Venugopal R, Jaiswal a K. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 1998;**17**:3145–56. doi:10.1038/sj.onc.1202237
- 15 Itoh K, Ishii T, Wakabayashi N, *et al.* Regulatory mechanisms of cellular response to oxidative stress. *Free Radic Res* 1999;**31**:319–24. doi:10.1080/10715769900300881
- 16 Zhao X, Song S, Sun G, *et al.* Neuroprotective Role of Haptoglobin after Intracerebral Hemorrhage. *J Neurosci* 2009;**29**:15819–27. doi:10.1523/JNEUROSCI.3776-09.2009
- 17 Galea J, Cruickshank G, Teeling JL, *et al.* The intrathecal CD163-haptoglobin-hemoglobin scavenging system in subarachnoid hemorrhage. *J Neurochem* 2012;**121**:785–92. doi:10.1111/j.1471-4159.2012.07716.x
- 18 Kristiansen M, Graversen JH, Jacobsen C, *et al.* Identification of the haemoglobin scavenger receptor. *Nature* 2001;**409**:198–201. doi:10.1038/35051594
- 19 Bulters D, Gaastra B, Zolnourian A, *et al.* Haemoglobin scavenging in intracranial bleeding: biology and clinical implications. *Nat Rev Neurol* 2018;**14**:416–32. doi:10.1038/s41582-018-0020-0
- 20 Zhao X, Sun G, Ting SM, *et al.* Cleaning up after ICH: The role of Nrf2 in modulating microglia function and hematoma clearance. *J Neurochem* 2015;**133**:144–52. doi:10.1111/jnc.12974
- 21 Morris CM, Candy JM, Edwardson JA, *et al.* Evidence for the localization of haemopexin immunoreactivity in neurones in the human brain. *Neurosci Lett* 1993;**149**:141–4. doi:10.1016/0304-3940(93)90756-B
- 22 Chen M, Regan RF. Time course of increased heme oxygenase activity and expression after experimental intracerebral hemorrhage: Correlation with oxidative injury. *J Neurochem* 2007;**103**:2015–21. doi:10.1111/j.1471-4159.2007.04885.x
- 23 Harada N, Kanayama M, Maruyama A, *et al.* Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages. *Arch Biochem Biophys* 2011;**508**:101–9. doi:10.1016/j.abb.2011.02.001

- 1
2
3
4 24 Sandberg M, Patil J, D'Angelo B, *et al.* NRF2-regulation in brain
5 health and disease: Implication of cerebral inflammation.
6 *Neuropharmacology* 2014;**79**:298–306.
7 doi:10.1016/j.neuropharm.2013.11.004
8
9 25 Pan H, Wang H, Zhu L, *et al.* Depletion of Nrf2 enhances
10 inflammation induced by oxyhemoglobin in cultured mice
11 astrocytes. *Neurochem Res* 2011;**36**:2434–41. doi:10.1007/s11064-
12 011-0571-6
13
14 26 Chen G, Fang Q, Zhang J, *et al.* Role of the Nrf2-ARE pathway in
15 early brain injury after experimental subarachnoid hemorrhage. *J*
16 *Neurosci Res* 2011;**89**:515–23. doi:10.1002/jnr.22577
17
18 27 Li T, Wang H, Ding Y, *et al.* Genetic elimination of Nrf2
19 aggravates secondary complications except for vasospasm after
20 experimental subarachnoid hemorrhage in mice. *Brain Res*
21 2014;**1558**:90–9. doi:10.1016/j.brainres.2014.02.036
22
23 28 Zhao XD, Zhou YT, Lu XJ. Sulforaphane enhances the activity of
24 the Nrf2-ARE pathway and attenuates inflammation in OxyHb-
25 induced rat vascular smooth muscle cells. *Inflamm Res*
26 2013;**62**:857–63. doi:10.1007/s00011-013-0641-0
27
28 29 Zhao X, Wen L, Dong M, *et al.* Sulforaphane activates the cerebral
29 vascular Nrf2–ARE pathway and suppresses inflammation to
30 attenuate cerebral vasospasm in rat with subarachnoid hemorrhage.
31 *Brain Res* 2016;**1653**:1–7. doi:10.1016/j.brainres.2016.09.035
32
33 30 Durnford A, Dunbar J, Galea J, *et al.* Haemoglobin scavenging after
34 subarachnoid haemorrhage. *Acta Neurochir Suppl* 2015;**120**:51–4.
35 doi:10.1007/978-3-319-04981-6_9
36
37 31 Farrell B, Godwin J, Richards S, *et al.* The United Kingdom
38 transient ischaemic attack (UK-TIA) aspirin trial: Final results. *J*
39 *Neurol Neurosurg Psychiatry* Published Online First: 1951.
40 doi:10.1136/jnnp.54.12.1044
41
42 32 Rankin J. Cerebral Vascular Accidents in Patients over the Age of
43 60: III. Diagnosis and Treatment. *Scott Med J* Published Online
44 First: 1957. doi:10.1177/003693305700200604
45
46 33 Quinn TJ, Dawson J, Walters MR, *et al.* Reliability of the modified
47 rankin scale: A systematic review. *Stroke* Published Online First:
48 2009. doi:10.1161/STROKEAHA.109.557256
49
50 34 Jennett B, Bond M. ASSESSMENT OF OUTCOME AFTER
51 SEVERE BRAIN DAMAGE. A Practical Scale. *Lancet* Published
52 Online First: 1975. doi:10.1016/S0140-6736(75)92830-5
53
54 35 Jennett B, Snoek J, Bond MR, *et al.* Disability after severe head
55 injury: Observations on the use of the Glasgow Outcome Scale. *J*
56 *Neurol Neurosurg Psychiatry* Published Online First: 1981.
57 doi:10.1136/jnnp.44.4.285
58
59
60

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2
3
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51
52
53
54
55
56
57
58
59
60
- 36 Pace A, Mitchell S, Casselden E, *et al.* A subarachnoid haemorrhage-specific outcome tool. *Brain* 2018;**141**:1111–21. doi:10.1093/brain/awy003
- 37 Powell JH, Beckers K, Greenwood RJ. Measuring progress and outcome in community rehabilitation after brain injury with a new assessment instrument - The BICRO-39 scales. *Arch Phys Med Rehabil* Published Online First: 1998. doi:10.1016/S0003-9993(98)90265-9
- 38 van Heugten C, Rasquin S, Winkens I, *et al.* Checklist for cognitive and emotional consequences following stroke (CLCE-24): development, usability and quality of the self-report version. *Clin Neurol Neurosurg* 2007;**109**:257–62. doi:10.1016/j.clineuro.2006.10.002
- 39 Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. *Neurosurgery* 1980;**6**:1–9. <http://www.ncbi.nlm.nih.gov/pubmed/7354892>
- 40 Naidech AM, Janjua N, Kreiter KT, *et al.* Predictors and impact of aneurysm rebleeding after subarachnoid hemorrhage. *Arch Neurol* 2005;**62**:410–6. doi:10.1001/archneur.62.3.410
- 41 Zhao J, Kobori N, Aronowski J, *et al.* Sulforaphane reduces infarct volume following focal cerebral ischemia in rodents. *Neurosci Lett* 2006;**393**:108–12. doi:10.1016/j.neulet.2005.09.065
- 42 Zhao X, Sun G, Zhang J, *et al.* Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. *Stroke* 2007;**38**:3280–6. doi:10.1161/STROKEAHA.107.486506
- 43 Alfieri A, Srivastava S, Siow RCM, *et al.* Sulforaphane preconditioning of the Nrf2/HO-1 defense pathway protects the cerebral vasculature against blood-brain barrier disruption and neurological deficits in stroke. *Free Radic Biol Med* 2013;**65**:1012–22. doi:10.1016/j.freeradbiomed.2013.08.190
- 44 Sharma V, McNeill JH. To scale or not to scale: The principles of dose extrapolation. *Br. J. Pharmacol.* 2009. doi:10.1111/j.1476-5381.2009.00267.x
- 45 Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* Published Online First: 2007. doi:10.1096/fj.07-9574LSF
- 46 Clarke JD, Hsu A, Williams DE, *et al.* Metabolism and tissue distribution of sulforaphane in Nrf2 knockout and wild-type mice. *Pharm Res* 2011;**28**:3171–9. doi:10.1007/s11095-011-0500-z
- 47 Jazwa A, Rojo AI, Innamorato NG, *et al.* Pharmacological Targeting of the Transcription Factor Nrf2 at the Basal Ganglia Provides Disease Modifying Therapy for Experimental

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42
43
44
45
46
47
48
49
50
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55
56
57
58
59
60
- Parkinsonism. *Antioxid Redox Signal* 2011;**14**:2347–60.
doi:10.1089/ars.2010.3731
- 48 Kumar G, Shahripour RB, Harrigan MR. Vasospasm on transcranial
Doppler is predictive of delayed cerebral ischemia in aneurysmal
subarachnoid hemorrhage: a systematic review and meta-analysis.
2016;**124**:1257–64. doi:10.3171/2015.4.JNS15428.
- 49 Tam AKH, Ilodigwe D, Li Z, *et al.* Global cerebral atrophy after
subarachnoid hemorrhage: a possible marker of acute brain injury
and assessment of its impact on outcome. *Acta Neurochir Suppl*
Published Online First: 2013. doi:10.1007/978-3-7091-1192-5_5
- 50 Campbell N, Verschuur C, Mitchell S, *et al.* Hearing impairment
after subarachnoid haemorrhage. *Ann Clin Transl Neurol* 2018;**In**
press. doi:10.1002/acn3.714
- 51 Vergouwen MDI, Vermeulen M, van Gijn J, *et al.* Definition of
delayed cerebral ischemia after aneurysmal subarachnoid
hemorrhage as an outcome event in clinical trials and observational
studies: proposal of a multidisciplinary research group. *Stroke*
2010;**41**:2391–5. doi:10.1161/STROKEAHA.110.589275
- 52 Alaraj A, Hussein AE, Esfahani DR, *et al.* Reducing length of stay
in aneurysmal subarachnoid hemorrhage: A three year institutional
experience. *J Clin Neurosci* 2017;**42**:66–70.
doi:10.1016/j.jocn.2017.03.049
- 53 Teasdale GM, Drake CG, Hunt W, *et al.* A universal subarachnoid
haemorrhage scale: report of a committee of the world federation of
Neurosurgical societies. *J. Neurol. Neurosurg. Psychiatry*. 1988.
doi:10.1136/jnnp.51.11.1457
- 54 Jaja BNR, Saposnik G, Lingsma HF, *et al.* Development and
validation of outcome prediction models for aneurysmal
subarachnoid haemorrhage: The SAHIT multinational cohort study.
BMJ Published Online First: 2018. doi:10.1136/bmj.j5745

BMJ Open

A study protocol for SFX-01 After Subarachnoid haemorrhage (SAS): A multi-centre randomised double-blinded, placebo controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-028514.R1
Article Type:	Protocol
Date Submitted by the Author:	23-May-2019
Complete List of Authors:	Zolnourian, Ardalan; University of Southampton, Clinical Neuroscience; University Hospital Southampton NHS Foundation Trust, Galea, Ian; University of Southampton Faculty of Medicine, Experimental Neurology Bulters, Diederik ; University Hospital Southampton NHS Foundation Trust
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Medical management, Evidence based practice, Neurology, Surgery, Pharmacology and therapeutics
Keywords:	Randomised controlled trial, Subarachnoid haemorrhage, Nrf2, Sulforaphane, Delayed cerebral ischaemia

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4 1 **A study protocol for SFX-01 After Subarachnoid haemorrhage**
5 2 **(SAS): A multi-centre randomised double-blinded, placebo**
6 3 **controlled trial**
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10 6 **Date and version:** 16th May 2018, Version 7
11 7

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1
2
3 **Abstract**
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5 51

6 **Introduction**
7

8 53

9 54 Subarachnoid haemorrhage from a ruptured cerebral aneurysm carries high morbidity
10 55 and mortality. Despite huge advances in techniques to secure the aneurysm, there has
11 56 been little progress in the treatment of the deleterious effects of the haemorrhage.
12 57

13 58

14 59 Sulforaphane is an Nrf2 inducer with anti-oxidant and anti-inflammatory properties. It
15 60 has been shown to improve clinical outcome in experimental models of SAH, but is
16 61 unstable. SFX-01 (Evgen Pharma) is a novel composition comprised of synthetic
17 62 sulforaphane stabilised within an α -cyclodextrin complex. On ingestion, the complex
18 63 releases sulforaphane making SFX-01 an ideal vehicle for delivery of sulforaphane.

19 64 **Methods and analysis**
20

21 65

22 66 The objective of the study is to assess the safety, pharmacokinetics and efficacy of
23 67 SFX-01. This is a prospective, multi-centre, randomised, double-blind placebo-
24 68 controlled trial in patients aged 18-80 years with aneurysmal subarachnoid
25 69 haemorrhage in the previous 48 hours. 90 patients will be randomised to receive SFX-
26 70 01 (300mg) or placebo twice-daily for up to 28 days.
27 71

28 72

29 73 Safety will be assessed using blood tests and adverse event reporting.
30 74 Pharmacokinetics will be assessed based on paired blood and CSF sulforaphane levels
31 75 on day seven. A subgroup will have hourly samples taken during six hours post-
32 76 dosing on days one and seven. Pharmacodynamics will be assessed by haptoglobin
33 77 and malondialdehyde levels, and maximum flow velocity of middle cerebral artery
34 78 will be measured by transcranial Doppler ultrasound.

35 79

36 80 Clinical outcomes will be assessed at days 28, 90 and 180 with mRS, GOSE, SAHOT,
37 81 SF-36, BICRO-39 and CLCE-24. MRI at six months including quantitative
38 82 susceptibility mapping and volumetric T1 will measure iron deposition and cortical
39 83 volume.
40 84

41 85

42 86 Safety, CSF sulforaphane concentration and middle cerebral artery flow velocity will
43 87 be primary outcomes and all others secondary.
44 88

45 89

46 **Ethics and dissemination**
47

48 90

49 91 Ethical approval was obtained from South Central Hampshire A committee.
50 92 Outcomes of the trial will be submitted for publication in a peer reviewed journal.
51 93

52 94

53 95 **Trial registration number:** NCT02614742 ; Pre-results
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96 **Strengths and limitations of this study**

- 97 • **A strength of this study is that it tests a new class of drug not previously used**
- 98 **after human SAH.**
- 99 • **It is at low risk of bias due to placebo control and appropriate blinding.**
- 100 • **The study design includes multiple mechanistic outcomes to give deeper**
- 101 **understanding of any clinical findings.**
- 102 • **The study includes multiple novel outcome measures and MRI sequences.**
- 103 **While these may provide new insights, some are exploratory due to limited**
- 104 **experience with them in SAH to date.**
- 105 • **It is a multicentre trial, and its results should be generalizable to patients**
- 106 **with high volume SAH in neurosurgical units in the UK, although due to the**
- 107 **complexity of its design, it has been limited to three centres to ensure high**
- 108 **quality outcome measurement.**

110 **Introduction**

111 Spontaneous Subarachnoid Haemorrhage (SAH) is a devastating cerebrovascular
112 injury with an incidence of 9.1 per 100,000 population [1]. It affects around 7000
113 patients in the UK annually. Around 85% are due to ruptured intracranial aneurysms
114 [2]. The incidence is age related peaking at 52 years. SAH carries a high overall
115 mortality rate of up to 67% [3], and only half of the survivors are able to live
116 independently [4]. It therefore has a high burden on society due to the loss of
117 productivity and resources [5].

118
119 Conventionally following SAH, treatment is primarily directed to securing the
120 aneurysm and prevent further re-bleeding. This however does nothing to ameliorate
121 the morbidity and mortality due to the haemorrhage. The only approved treatment is
122 nimodipine [6]. However, its effects are small and poor outcome remains a significant
123 problem [7]. Moreover, even in survivors considered to have made a good recovery,
124 neurocognitive deficits are common leading to extensive problems with social
125 reintegration and functioning in the workplace [5].

126
127 The mechanism of injury following SAH is multifactorial[8]. Early brain injury (EBI)
128 refers to the processes occurring within the first 72 hours which include blood-brain
129 barrier (BBB) dysfunction [9], cerebral oedema [10][11], neuronal cell death [12],
130 altered ionic homeostasis, excitotoxicity, thrombin activation [13], vascular integrity
131 degradation [14], oxidative stress [15], and inflammation [16]. However, despite the
132 terminology, mechanisms such as oxidative stress and inflammation are not limited to
133 this early period. They continue to worsen beyond the first three days, at the same
134 time as CSF free haemoglobin (Hb) concentration rises markedly as it is released
135 from the clot and mechanisms to dispose of Hb are saturated. It is also in this delayed
136 phase when cerebral vasospasm occurs, affecting both micro- [17] and
137 microvasculature [18].

138
139 Sulforaphane (SFN) is known to upregulate the nuclear factor erythroid 2-related
140 factor 2 (Nrf2) pathway. Nrf2 is a redox-sensitive transcription factor that binds to a
141 specific DNA site, the anti-oxidant response element, upstream of genes encoding
142 detoxifying and anti-oxidant enzymes [19] [20]. Some of these enzymes include

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3 143 glutathione S-transferases (GSTs), NAD(P)H-quinone oxidoreductase 1 (NQO1) and
4 144 haem oxygenase 1 (HO-1) [19]. During physiological conditions Nrf2 is bound to the
5 145 Kelch-like ECH associated protein 1 (KEAP1) in the cytoplasm. In response to stress
6 146 such as SAH, Nrf2 is released from KEAP1 and then translocates to the nucleus
7 147 leading to enhanced gene transcription [21].
8 148

9 149 Nrf2 also upregulates haptoglobin (Hp), an acute phase glycoprotein found in plasma
10 150 [22], as well as cerebrospinal fluid (CSF) [23]. Hp is part of an important Hb
11 151 scavenging pathway after SAH. It binds tightly to free Hb, and this Hb-Hp complex is
12 152 taken up by CD163-positive macrophages [24]. This pathway is saturated after SAH
13 153 [23] and upregulation of Hp represents a possible therapeutic avenue [25]. Nrf2 also
14 154 regulates degradation of red blood cells, and metabolism of haem and iron through
15 155 transcriptional upregulation of CD36 [26], haemopexin [27], HO-1 [28] and ferritin
16 156 [29].
17 157

18 158 Nrf2 is expressed in the central nervous system (CNS) and is upregulated in response
19 159 to inflammation and cerebral insults [30]. Nrf2 knockout is associated with a more
20 160 pronounced inflammatory response *in vitro* [31] and *in vivo* [32], and the increased
21 161 inflammatory response is associated with more brain oedema, cell death and poorer
22 162 neurological recovery [33]. SFN increases HO-1, NQO1, and GST- α 1 levels and
23 163 reduces IL-1 β , IL-6, and TNF- α [34]. It leads to a reduction in vasospasm and
24 164 improves neurological recovery [32] [35].
25 165

26 166 SFN has a relatively short half-life rendering it impractical for clinical use. SFX-01
27 167 (Evgen Pharma) is a novel new agent comprising SFN complexed with α -cyclodextrin
28 168 which is suitable for clinical use. On ingestion, SFN is released from the α -
29 169 cyclodextrin and is an effective method to deliver SFN. In two Phase I trials
30 170 (NCT01948362, NCT02055716) no serious adverse events were reported in healthy
31 171 volunteers. Here we describe the protocol for a phase II trial of SFX-01 in patients
32 172 who have suffered a SAH.
33 173

34 174 **Objectives**

35 175
36 176 The objective of the study is to assess the safety, pharmacokinetics and efficacy of
37 177 SFX-01.
38 178

39 179 **Methods**

40 180 ***Trial design***

41 181
42 182
43 183 This is a prospective, double-blind, parallel group, randomised controlled trial
44 184 comparing SFX-01 (300 mg) taken orally as capsules or as a suspension via a
45 185 nasogastric tube (NG) twice-daily for up to 28 days *versus* placebo in patients with
46 186 SAH within 48 hours of enrolment.
47 187

48 188 The treatment window of 48 hours was selected as the best compromise between
49 189 competing factors. SFX-01 has pleiotropic actions against multiple mechanisms each
50 190 with different temporal profiles. Since SAH is an acute unpredictable event, the
51 191 earliest one would be able to start treatment is soon after ictus, on admission into

1
2
3 192 hospital. This would give optimal protection against early brain injury. Initiating
4 193 treatment would still be justified up to 72 hours post-ictus, after which delayed
5 194 cerebral ischaemia (DCI) occurs, such that a delay of more than 72 hours would be
6 195 expected to undermine treatment efficacy. Within the initial 72 hours, earlier
7 196 treatment would allow more time for Nrf2 pathway activation and expression of its
8 197 transcriptome to protect against delayed events, as well as provide more opportunity
9 198 for SFX-01 to act against early brain injury. On the other hand the earlier one
10 199 stipulates treatment would start, the more patients one will exclude from the study. No
11 200 animal studies have been conducted to investigate the timing of sulforaphane
12 201 administration, and even if available, extrapolation of timing from animal models has
13 202 its limitations due to the much quicker clot resorption in rodents. After considering all
14 203 these factors, it was decided to start SFX-01 treatment at the earliest possible
15 204 opportunity after SAH, yet still allow patients to be included if their presentation was
16 205 delayed to some extent, to ensure generalizability to real clinical practice where
17 206 delays in admission to tertiary centres are not uncommon. An audit of the lead study
18 207 centre showed that most patients are admitted within 48 hours, leading to the adoption
19 208 of SAH within 48 hours as the inclusion criterion, striking the best compromise
20 209 between the practicality of recruitment and the need to start treatment early.
21 210

22 211 Detailed pharmacovigilance will inform the safety of SFX-01. Pharmacokinetics will
23 212 be determined by measuring levels of SFN and its metabolites in CSF and blood at
24 213 day seven in all patients; a more detailed profile will be obtained in a subgroup of up
25 214 to 12 patients who will have hourly blood and CSF samples for six hours after dosing
26 215 on days one and seven. Pharmacodynamics will be assessed with blood Hp and
27 216 malondialdehyde (MDA) levels, and middle cerebral artery (MCA) flow velocity as
28 217 measured on transcranial Doppler (TCD) ultrasound as an estimate of large cerebral
29 218 artery spasm. Longer term outcome will be assessed using validated outcome scales,
30 219 at day 28 using Modified Rankin Scale (mRS) ([36] [37] [38] , Glasgow Outcome
31 220 Score (GOSE) [39][40] and the SAH Outcome Tool (SAHOT) [41] as well as at day
32 221 90 and 180 using the mRS, GOSE, SAHOT, Brain Injury Community Rehabilitation
33 222 Outcome Scales (BICRO-39) [42] and the Check List for Cognitive and Emotional
34 223 consequences following stroke (CLCE-24) [43].
35 224

36 225 *Patient and public involvement*

37 226
38 227 The overall design of the study including planned investigations i.e. lumbar puncture
39 228 (LP), magnetic resonance imaging (MRI), blood tests, outcome questionnaires, the
40 229 treatment methods and the consent procedures were discussed in a local SAH support
41 230 group consisting of individuals with a previous history of SAH as well as their
42 231 relatives. The meeting was led by the chief investigator, a consultant neurovascular
43 232 surgeon, and the neurovascular specialist nurse who are normally the main point of
44 233 contact for SAH patients. The results were used to inform planning of the trial.
45 234 Particular attention was given to the study lumbar puncture which was felt to be
46 235 justified on the grounds that the majority of patients will undergo CSF diversion for
47 236 clinical reasons anyway, and evidence from a randomised controlled trial that CSF
48 237 diversion in all Fisher 3&4 patients causes no harm and appears to provide short term
49 238 symptomatic benefit [44]. The meeting was also beneficial in shaping and improving
50 239 the patient information sheet and consent forms.
51 240

52 241 *Study setting*

242

This is a multicentre study conducted in regional neurosciences centres with specialist services to treat aneurysmal SAH. Patients will be identified at referral to the admitting neurosurgical or neurointensive care units. After informed consent, dosing and study interventions will occur in the neurosciences centre until patients are discharged to either home or their local hospitals. There are three recruiting centres; University Hospital Southampton, Royal Infirmary of Edinburgh and the Royal London Hospital. Upon discharge, follow-up may take place in clinic, or at visits to district general hospitals and rehabilitation centres, or patients' residences.

251

Eligibility Criteria

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Inclusion criteria

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1. Patients with radiological evidence of spontaneous aneurysmal SAH
2. Fisher grade 3 or 4 on CT
3. Definitive treatment of aneurysm has not been ruled out
4. Previously living independently
5. In the opinion of the investigator, the delay from ictus to randomisation and initiation of trial medication will not exceed 48 hours
6. Aged 18 to 80 years
7. In the opinion of the investigator it will be possible to obtain Informed Consent from the Patient, Personal Legal Representative or Professional Legal representative within 24 hours of first dose

267

Exclusion criteria

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Recruitment will be limited to Fisher grade 3 and 4 SAH. These patients represent the majority of aneurysmal SAH. They have a higher volume of haemorrhage with a poorer outcome and more delayed neurological deficits [45]. They are therefore mechanistically and clinically expected to derive greatest benefit from SFX-01.

291
292 Inclusion of patients with unsecured aneurysms would risk a high incidence of
293 rebleeding in the study. Rebleeding is associated with exceedingly bad outcomes [46],
294 masking any effect from SFX-01. However, since not all aneurysms are secured
295 within 48 hours, there will be no requirement for the aneurysm to have been secured
296 prior to enrolment. Instead patients in whom treatment of the aneurysm has been ruled
297 out due to poor clinical status will be excluded.

298
299 Although there are no known risks to kidney or liver, due to the relative inexperience
300 with SFX-01 in humans, patients with liver or kidney problems will be excluded.

301 302 **Intervention**

303 304 ***Trial medication***

305
306 SFX-01 (active 300 mg capsule) or placebo (α -cyclodextrin only capsule) will be
307 taken orally or as a suspension via a nasogastric tube (NG) twice daily for up to 28
308 days. Pharmagra manufacture the active pharmaceutical ingredient (API) in North
309 Carolina, US. The API is then encapsulated at Quotient in Reading, UK. The API
310 manufacture has been audited by the FDA and Evgen pharma. SFX-01 and placebo
311 capsules as well as their contents will be identical in appearance. They will be stored
312 at 2-8 degrees Celsius.

313
314 Animal studies in ischaemic stroke, intracerebral haemorrhage and SAH have all used
315 5 mg/kg dose of SFN in rodents [32] [47] [48] [49]. Conversion of animal doses to
316 humans using body surface area, as has been widely recommended [50] [51], yields a
317 human dose of 50 mg SFN. This is equivalent to 300 mg of SFX-01 containing 46.15
318 mg of SFN. In the clinical studies conducted to date, SFX-01 has been shown to be
319 well tolerated at doses of 600mg once daily and 300mg twice daily with no serious
320 adverse effects. Therefore, no further dose ranging studies were performed.

321 322 ***De-escalation from the trial regimen***

323
324 In the event of tolerability problems whilst the patient is in the neurosurgical centre,
325 the Investigator will assess whether simple measures to ease the effects of the adverse
326 event(s) may be implemented (i.e. antacid in the case of GI irritation or anti-emetic in
327 the event of nausea).

328 The investigator will also assess whether or not the adverse event(s) could be related
329 to the trial medication and severe enough to warrant a dose frequency reduction. In
330 the first instance the investigator may consider missing one dose. If a dose frequency
331 reduction is warranted, from that point onwards the second dose of the day will be
332 omitted; a dose frequency increase back to twice daily will not be permitted.

333
334 If tolerability problems continue then the investigational medication will be stopped;
335 patients will continue in the study and complete the study visits. The staged dose
336 frequency de-escalation (dropping to once daily) will not be carried out after
337 discharge from the neurosurgical centre; if tolerability problems occur after discharge,
338 medication will be stopped; patients will continue in the study and complete the study
339 visits in accordance with the schedule of assessments.

340

1
2
3 341 ***Treatment compliance***

4 342
5 343 Compliance with treatment will be recorded during the inpatient hospital stay by
6 344 health care professionals and/or a member of the research team. On discharge to the
7 345 usual residence, responsibility for this will be transferred to the patient or their
8 346 Personal Legal Representative, aided by detailed instructions. In the event of
9 347 discharge to a rehabilitation unit or patient local hospital, written instructions will be
10 348 provided on discharge and verbal communication with the clinical team will ensure
11 349 compliance.

12 350
13 351 All patients will be discharged with a patient diary which will be filled in and
14 352 collected at day 28. Compliance will be further monitored by drug reconciliation.
15 353 Patients will be asked to return the medication bottle and any residual contents at the
16 354 day 28 visit. At this time any residual tablets will be counted and recorded.

17 355
18 356 ***Concomitant treatment***

19 357
20 358 There are no known drug interactions, and participation in the trial will not alter
21 359 routine treatment of SAH. Participation in other interventional research studies will
22 360 not be allowed until after the last follow up visit.

23 361
24 362 **Outcomes**

25 363
26 364 **Primary end-points**

27 365
28 366 ***Safety***

29 367
30 368 To evaluate the safety of up to 28 days of SFX-01 dosed at up to an equivalent of
31 369 92mg SFN per day.

32 370
33 371 Prior to the start of this study, the trial medication had only been used in healthy
34 372 individuals and not in a patient population. Therefore safety will be one of the main
35 373 objectives of this study. This will be evaluated through routine tests (full blood count,
36 374 urea and electrolytes, coagulation screen, liver function tests, and urine microscopy)
37 375 at baseline, post-dose, day seven and day 28 as well as close monitoring of patients
38 376 for any side-effects or adverse events. Adverse events will be coded following
39 377 MEDDRA and followed up until resolution and graded for severity. Incidence of dose
40 378 de-escalation or discontinuation will also be reported.

41 379
42 380 ***Pharmacokinetic***

43 381
44 382 To detect the presence of SFN and its metabolites in CSF and blood.

45 383
46 384 Animal models have shown that SFN crosses the blood-brain barrier and can therefore
47 385 be detected in the brain [52] [53] There is some variation in the levels achieved in
48 386 these studies, and it has not been studied in humans. All patients will have a paired
49 387 CSF/blood sample taken at seven days post-ictus. This will be via a LP unless the
50 388 patient has an external ventricular drain (EVD) for their clinical care in which case it
51 389 will be obtained from the EVD. In addition, up to 12 patients with an EVD will be
52 390 asked to consent to hourly CSF and blood samples for six hours after dosing on days

391 one and seven. Hourly CSF sampling will be performed by trained study personnel
392 using a bespoke sterile closed cascade of syringes so that the EVD line is accessed
393 directly only once to reduce the risk of infection. Sample collection and processing
394 are detailed in specific study operating procedures.

395

396 *Vasospasm*

397

398 To determine if a minimum of seven days treatment with SFX-01 reduces MCA peak
399 flow velocity following SAH.

400

401 TCD ultrasound will be used to measure the MCA, ICA and ECA maximum flow
402 velocity and the Lindegaard ratio will be calculated. TCDs will be performed on
403 alternate daily basis including at baseline. They will be performed for at least seven
404 days or until no longer clinically indicated. Blood flow velocity is measured in
405 cm/second and is inversely related to the luminal diameter of the vessel. The greater
406 this value the more likely the degree of narrowing or spasm in the vessel. It has a
407 good predictive value for DCI. A recent systematic review and meta-analysis, which
408 pooled data from 2870 patients, showed a sensitivity of 90%, specificity of 71%, and
409 negative and positive predictive values of 92% and 57% respectively [54].

410

411 Secondary end-points

412

413 *Pharmacodynamic*

414

415 *CSF and blood – Haptoglobin and Malondialdehyde*

416

417 Hp represents a major Hb detoxification pathway and is upregulated by Nrf2. MDA is
418 a measure of oxidative stress. Hp and MDA will be measured in blood at baseline,
419 day seven and day 28, and in CSF on day seven. A local audit at the main study
420 neurosurgical centre showed that approximately 1/3 of patients have an EVD sited as
421 part of their routine clinical care to treat hydrocephalus. In these patients additional
422 samples will be obtained. This will allow investigation of the temporal profile of Hp
423 level and oxidative stress. Additionally, there will be exploratory investigations using
424 proteomics, transcriptomics and genomics using CSF and blood samples.

425

426 *MRI – iron and brain volume*

427

428 All patients will have MRI 180 days after SAH. Brain volume on T1 sequences will
429 be measured, since this has been shown to correlate with outcome [55]. Cortical iron
430 content will be assessed using quantitative susceptibility mapping after susceptibility
431 weighted MR imaging, which predominantly measures siderotic iron deposits [56].
432 Iron is a major component of Hb, and it is unknown what effects SFX-01, SFN or
433 increased Hp binding of Hb may have on the downstream iron pathway.

434

435 *Clinical outcome*

436

437

438

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440

- mRS at seven days, discharge, 28, 90 and 180 days.
- Incidence of DCI defined as a new focal deficit or reduction in GCS (by two points or more) if not explained by other causes (i.e. re-bleed, hydrocephalus, seizure, meningitis, sepsis or hyponatraemia) [57]

- 1
2
3 441 • Incidence of new cerebral infarct on CT or MRI
4 442 • Institution of hypertensive therapy for presumed DCI
5 443 • SF-36 quality of life survey at 28, 90 & 180 days
6 444 • CLCE-24 and BICRO-39 at 90 & 180 days
7 445 • SAHOT and GOSE at 28, 90 & 180 days
8 446 • Length of acute hospital stay
9 447 • Discharge destination
10
11
12

13 449 A number of measures of efficacy have been selected reflecting the most common
14 450 stroke outcome assessment (mRS), the most common brain injury assessment
15 451 (GOSE), the most common quality of life survey (SF-36) and the only SAH specific
16 452 outcome tool [41] to determine the most sensitive tool and make estimates of effect
17 453 size. SAHOT includes 56 items dealing with cognitive, physical, and
18 454 behavioural/psychological consequences of SAH, developed in a SAH focus group by
19 455 patients and experts in the field. This outcome tool has been validated and proposed
20 456 as a more sensitive and responsive tool in SAH [41]. All these tools will be
21 457 administered by research nurses or doctors trained in mRS and GOSE in person, or in
22 458 the event this is not feasible, by phone.
23
24 459

25 460 A number of short term patient outcomes related to the incidence of DCI are included
26 461 *a priori* such as incidence of infarction as adjudicated by a blinded consultant
27 462 neuroradiologist (with baseline CT and follow up MRI), and institution of
28 463 hypertensive therapy. Several simple outcomes including length of stay and discharge
29 464 destination that have been associated with outcome [58] are also included *a priori* in
30 465 the event any patients are lost to longer term follow up.
31
32 466

33 467 Schedule of assessments

34 468

Time points ¹	D 0-2	D 1-3 (12-24 post- dose)	Ongoing assessment (Alternate days) ⁶ (+/-1)	D 7 (+/-1)	Discharge (-2)	D 28 (-6/+2)	D 90 (+/-14)	D 180 (+/- 28)
Study procedures								
Consent	X							
Inclusion/exclusion	X							
WFNS grading	X							
IMP treatment	X							
Safety bloods ²	X	X	X	X	X	X		
Safety urine	X			X	X	X		
Lipid profile Coagulation screen	X			X	X	X		
TCD readings ³		X	X	X				
HP, MDA (Blood/CSF) ⁴	X			X		X		
SFN & metabolites (Blood/CSF) ⁵				X				
Pregnancy test	X							
mRS				X	X	X	X	X
GOSE						X	X	X
SAHOT						X	X	X
SF-36						X	X	X

BICRO-39							X	X
CLCE-24							X	X
MRI								X

- 1- Ictus is defined as the onset of symptoms/haemorrhage and is referred to as day 0.
- 2- Safety bloods include: Biochemistry: Sodium, Potassium, Urea, Creatinine, Glucose, Calcium, Total Bilirubin, Alkaline Phosphatase, Alanine Transaminase, Albumin, C-Reactive Protein, and Haematology including Haemoglobin, White Blood Cell Count, Neutrophils (Absolute), Lymphocytes (Absolute), Platelets. These will be done at least on alternate days until no longer clinically indicated.
- 3- TCDs will be performed at baseline before day 3 and will be repeated on alternate daily basis until at least day 7 or where clinically indicated.
- 4- Hp and MDA will be assayed in both CSF and blood at baseline, where possible i.e if patient has an EVD fitted this will be measured in the CSF and blood at baseline as well as every other day until EVD is removed. All patients will have pP and MDA assayed on either a LP or EVD sample on day 7.
- 5- SFN and its metabolites will be measured on day 7 in all patient with paired blood and CSF (LP or EVD sample).
- 6- These assessments will be done on every other day basis with a +/- 1 day window. They will be carried on until discharge or up to when it is clinically required.

Sample Size

No formal sample size calculation has been carried out; the power associated with a sample size of 90 is based on the following assumptions:

- The error probability for the Type I error should not exceed 5% for a one-sided test;
- The primary endpoint will be compared between treatment groups by means of a t-test
- The mean maximum MCA flow velocity for patients treated with SFX-01 is estimated as 175 cm/s and
- The standard deviation of maximum MCA flow velocity is 50 cm/s

Under these assumptions 90 patients will give 80% power to detect a difference in maximum MCA velocity which is approximately half of the standard deviation of the mean value. The standard deviation was assumed to be approximately 30% of the mean value.

Recruitment

Patients with SAH who present to the clinical centres and meet the above criteria will be considered for recruitment. The inclusion and exclusion criteria reflect national practice. Patients will be identified by the treating clinical team at the time of referral, admission or daily medical handover.

Assignment of intervention

Randomisation and blinding

Patients will be randomised in a 1:1 ratio to the active or placebo arm. Randomisation will be stratified using the most recent World Federation of Neurosurgical Societies (WFNS) grade [59] prior to randomization. Patients in different WFNS groups will have significantly different outcomes [60] and imbalance between treatment arms risks treatment allocation bias.

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2
3 518
4 519 All treatment packs will be otherwise identical in appearance. Placebo capsules will
5 520 be identical and contain α -cyclodextrin making the contents indistinguishable should
6 521 they be opened either inadvertently or for the purposes of NG administration. Patients
7 522 will be randomised to one of the treatment groups by allocation of the appropriate,
8 523 sequentially numbered treatment pack from either the clinical trials pharmacy, or a
9 524 suitably calibrated and monitored fridge outside the pharmacy. The treatment packs
10 525 will be pre-numbered according to a block balanced randomisation code with a ratio
11 526 of 1:1 by a blinded third party. They will be selected as per WFNS grading by a
12 527 member of a research team from pharmacy.
13
14
15 528

16 529 ***Unblinding***

17 530
18 531 The Pharmacy will receive a sealed envelope containing the identity of each trial
19 532 medication bottle. An envelope may be opened only in the case of a serious adverse
20 533 event and only when it is essential to the subsequent management of the patient. The
21 534 decision to unblind will be made in discussion between the treating clinician and PI
22 535 and where possible CI. The independent trial centre will be responsible for breaking
23 536 codes for regulatory submissions of Suspected Unexpected Serious Adverse
24 537 Reactions (SUSARs), thereby maintaining the overall confidentiality of the code
25 538 breaks. If the code is broken the data for that patient will be excluded from the Per
26 539 Protocol Population analysis but included in the Intention to Treat Analysis. They will
27 540 continue in the study and complete the study visits in accordance with the study visit
28 541 schedule.
29
30
31 542

32 543 **Data collection and management**

33 544
34 545 Data collection will be performed by Good Clinical Practice-trained members of the
35 546 research team. Study specific training and additional training in disease specific
36 547 questionnaires will be provided. The data will be entered into a secure electronic case
37 548 report form.
38
39 549

40 550 ***Statistical analysis***

41 551
42 552 The following populations will be considered for the analysis:

- 43 553 • Intention-to-Treat population (ITT): all randomised patients who receive at
44 554 least one dose of study medication and with any post-dose efficacy
45 555 evaluations. Patients where the time from ictus to admission is unknown are
46 556 to be considered as part of the ITT population .
- 47 557 • Per-protocol population (PPP): The Per Protocol Population (for Primary
48 558 analysis) will be considered to be those patients in the ITT population that
49 559 have been dosed for a minimum to day seven post ictus without any major
50 560 protocol violations (i.e. wrong inclusions, etc.).
- 51 561 • Safety population: all randomised patients who have taken at least one dose of
52 562 study medication.
53
54
55 563

56 564 Last observation carried forwards (LOCF) will be used to impute missing outcome at
57 565 6 months. The final full statistical analysis plan will be published prior to unblinding.
58
59 566
60

567 ***Monitoring***

568
569 A data safety monitoring board (DSMB) has been set-up to monitor the safety aspect
570 of the trial throughout. The DSMB will be independent of the study team and
571 company and has its own charter. A steering committee (consisting of the Chief
572 Investigator and the sponsor's Chief Medical Officer) will receive and review the
573 reports from DSMB, and take action as appropriate.

574
575 The first 20 patients will only be dosed as an inpatient and may therefore have courses
576 shorter than 28 days. The DSMB plan to hold an initial meeting after recruitment of
577 20 patients who will have completed seven days of trial medication. If there are no
578 safety concerns, further patients will be allowed to be dosed with the trial drug after
579 discharge to other hospitals or home. The DSMB will also meet if there are any
580 SUSARs or if two patients have had an increase in the grading of the severity of
581 adverse events.

582
583 Recruitment will stop once the target has been reached or if DSMB deems the study
584 or trial drug to be associated with a significant number of adverse events compared to
585 the normal patient population. The recruitment target is set to a minimum of 90
586 patients between three centres in the United Kingdom. Up to 120 patients may be
587 recruited in order to allow for withdrawals and deaths.

588
589 External monitoring will occur regularly throughout the study and after the study has
590 been completed. At these visits the monitor(s) will inspect various study records; case
591 report forms, investigator site file and source data, provided that subject
592 confidentiality is respected.

593 594 ***Adverse events reporting***

595
596 All adverse events, adverse drug reactions and serious adverse events (SAE) will be
597 accurately documented. SAEs will be reported within 24 hours of awareness.
598 Pregnancies occurring during the study must be reported immediately and monitored
599 closely.

600 601 ***Data Availability***

602
603 Upon completion of the study, data will be shared with other eligible investigators
604 through academically established means. The datasets used and/or analysed during the
605 study will be available from the corresponding author on reasonable request.

606 607 **Ethical considerations and Informed Consent**

608 609 ***Consent procedures and emergency dosing***

610
611 Most patients with acute SAH present with either severe headache or altered level of
612 consciousness. Many will lack capacity with no legal representative immediately
613 available. SAH is an acute emergency and any benefit from SFX-01 is likely to be
614 greatest the earlier it is administered. The study has therefore been granted ethical
615 permission to obtain baseline blood testing and administer two doses of trial drug
616 without consent if the patient is lacking in capacity and no legal representative is

617 available. If no consent can be obtained at that point, the patient will be withdrawn
618 from the study.

619

620

621 Consent will be obtained in one of three scenarios:

622

623 1- Patients with capacity

624 2- Patients without capacity, but with a relative or next of kin available
625 immediately in person

626 3- Patients without capacity and no relative or next of kin immediately available
627 in person. In this case, a professional legal representative will be approached.

628

629 ***Re-Consent***

630

631 This must take place in two different scenarios:

632 1- When patients regain capacity they must be re-consented.

633 2- When patients have been consented through a professional legal
634 representative, after which time either they regain capacity or the next of kin
635 becomes available.

636

637 **Discussion**

638

639 There is mounting evidence supporting the role of the Nrf2 pathway in outcome after
640 SAH. SFN upregulates Nrf2 expression and improves outcome in animal models.
641 SFX-01 represents an exciting and novel way to deliver SFN to SAH patients with the
642 potential to improve their lives.

643

644 This trial will investigate the safety, pharmacokinetics and pharmacodynamics of
645 SFX-01 after SAH. If successful it may deliver long-term benefit to patients who have
646 suffered SAH and provide new hope to a group of patients characterised by complex
647 neurocognitive problems and disability.

648

649 **Footnotes**

650 **Authors' contributions:** DB conceived the trial. AZ, IG and DB were all involved in
651 the design of the study and its setup. AZ and DB wrote the study protocol. DB
652 managed the recruitment of other centres. IG and DB reviewed the protocol
653 manuscript and approved the final version.

654

655 **Acknowledgements:** The protocol was revised and finalised in conjunction with
656 David Howatt and Robert Holland previously at Evgen Pharma. The manuscript was
657 reviewed by Sally Ross and Thomas Morris of Evgen Pharma.

658

659 **Funding:** This study is funded and sponsored by EvgenPharma.

660

661 **Competing interests:** None declared. The authors have no financial or non financial
662 interests in Evgen Pharma.

663

664 **Ethical approval:** South Central Research Ethics Committee, Hampshire A, UK.

665 **Word count:** 4720

References

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667
668 1 de Rooij NK, Linn FHH, van der Plas JA, *et al.* Incidence of
669 subarachnoid haemorrhage: a systematic review with emphasis on
670 region, age, gender and time trends. *J Neurol Neurosurg Psychiatry*
671 2007;**78**:1365–72. doi:10.1136/jnnp.2007.117655
- 672 2 Van Gijn J, Rinkel GJE. Subarachnoid haemorrhage: Diagnosis,
673 causes and management. *Brain*. 2001. doi:10.1093/brain/124.2.249
- 674 3 Nieuwkamp DJ, Setz LE, Algra A, *et al.* Changes in case fatality of
675 aneurysmal subarachnoid haemorrhage over time, according to age,
676 sex, and region: a meta-analysis. *Lancet Neurol* 2009;**8**:635–42.
677 doi:10.1016/S1474-4422(09)70126-7
- 678 4 Kirkpatrick P, Lindsay K, Shaw M, *et al.* *National Study of*
679 *Subarachnoid Haemorrhage*. 2006.
- 680 5 Rivero-Arias O, Gray A, Wolstenholme J. Burden of disease and
681 costs of aneurysmal subarachnoid haemorrhage (aSAH) in the
682 United Kingdom. *Cost Eff Resour Alloc* 2010;**8**:1–12.
683 doi:10.1186/1478-7547-8-6
- 684 6 Pickard JD, Murray GD, Illingworth R, *et al.* Effect of oral
685 nimodipine on cerebral infarction and outcome after subarachnoid
686 haemorrhage : British aneurysm nimodipine trial. *Bmj*
687 1989;**298**:636–42. doi:10.1136/bmj.298.6674.636
- 688 7 Dorhout Mees SM, Rinkel GJ, Feigin VL, *et al.* Calcium
689 antagonists for aneurysmal subarachnoid haemorrhage. *Cochrane*
690 *Database Syst Rev* Published Online First: 2007.
691 doi:10.1002/14651858.CD000277.pub3
- 692 8 Zolnourian A, Galea I, Bulters D. Neuroprotective Role of the Nrf2
693 Pathway in Subarachnoid Haemorrhage and Its Therapeutic
694 Potential. *Oxid Med Cell Longev* 2019;**2019**:1–21.
695 doi:10.1155/2019/6218239
- 696 9 Dóczi T. The pathogenetic and prognostic significance of blood-
697 brain barrier damage at the acute stage of aneurysmal subarachnoid
698 haemorrhage. Clinical and experimental studies. *Acta Neurochir*
699 *(Wien)* 1985;**77**:110–
700 32.<http://www.ncbi.nlm.nih.gov/pubmed/4072781>
- 701 10 László FA, Varga C, Dóczi T. Cerebral oedema after subarachnoid
702 haemorrhage. Pathogenetic significance of vasopressin. *Acta*
703 *Neurochir (Wien)* 1995;**133**:122–
704 33.<http://www.ncbi.nlm.nih.gov/pubmed/8748754>
- 705 11 Zetterling M, Hallberg L, Ronne-Engström E. Early global brain
706 oedema in relation to clinical admission parameters and outcome in
707 patients with aneurysmal subarachnoid haemorrhage. *Acta*
708 *Neurochir (Wien)* 2010;**152**:1527–33. doi:10.1007/s00701-010-

- 1
2
3 709 0684-8
4
5 710 12 Cahill WJ, Calvert JH, Zhang JH. Mechanisms of early brain injury
6 711 after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*
7 712 2006;**26**:1341–53. doi:10.1038/sj.jcbfm.9600283
8
9 713 13 Lad SP, Hegen H, Gupta G, *et al.* Proteomic biomarker discovery in
10 714 cerebrospinal fluid for cerebral vasospasm following subarachnoid
11 715 hemorrhage. *J Stroke Cerebrovasc Dis* 2012;**21**:30–41.
12 716 doi:10.1016/j.jstrokecerebrovasdis.2010.04.004
13
14 717 14 Kusaka G, Ishikawa M, Nanda A, *et al.* Signaling pathways for
15 718 early brain injury after subarachnoid hemorrhage. *J Cereb Blood*
16 719 *Flow Metab* Published Online First: 2004.
17 720 doi:10.1097/01.WCB.0000125886.48838.7E
18
19 721 15 Gaetani P, Pasqualin A, Rodriguez y Baena R, *et al.* Oxidative
20 722 stress in the human brain after subarachnoid hemorrhage. *J*
21 723 *Neurosurg* Published Online First: 2009.
22 724 doi:10.3171/jns.1998.89.5.0748
23
24 725 16 Fassbender K, Hodapp B, Rossol S, *et al.* Inflammatory cytokines
25 726 in subarachnoid haemorrhage: Association with abnormal blood
26 727 flow velocities in basal cerebral arteries. *J Neurol Neurosurg*
27 728 *Psychiatry* Published Online First: 2001. doi:10.1136/jnnp.70.4.534
28
29 729 17 Pennings FA, Bouma GJ, Ince C. Direct observation of the human
30 730 cerebral microcirculation during aneurysm surgery reveals
31 731 increased arteriolar contractility. *Stroke* 2004;**35**:1284–8.
32 732 doi:10.1161/01.STR.0000126039.91400.cb
33
34 733 18 Dorsch NWC, King MT. A review of cerebral vasospasm in
35 734 aneurysmal subarachnoid haemorrhage Part I: Incidence and effects.
36 735 *J. Clin. Neurosci.* 1994. doi:10.1016/0967-5868(94)90005-1
37
38 736 19 Itoh K, Chiba T, Takahashi S, *et al.* An Nrf2/small Maf heterodimer
39 737 mediates the induction of phase II detoxifying enzyme genes
40 738 through antioxidant response elements. *Biochem Biophys Res*
41 739 *Commun* 1997;**236**:313–22. doi:10.1006/bbrc.1997.6943
42
43 740 20 Venugopal R, Jaiswal a K. Nrf2 and Nrf1 in association with Jun
44 741 proteins regulate antioxidant response element-mediated expression
45 742 and coordinated induction of genes encoding detoxifying enzymes.
46 743 *Oncogene* 1998;**17**:3145–56. doi:10.1038/sj.onc.1202237
47
48 744 21 Itoh K, Ishii T, Wakabayashi N, *et al.* Regulatory mechanisms of
49 745 cellular response to oxidative stress. *Free Radic Res* 1999;**31**:319–
50 746 24. doi:10.1080/10715769900300881
51
52 747 22 Zhao X, Song S, Sun G, *et al.* Neuroprotective Role of Haptoglobin
53 748 after Intracerebral Hemorrhage. *J Neurosci* 2009;**29**:15819–27.
54 749 doi:10.1523/JNEUROSCI.3776-09.2009
55
56 750 23 Galea J, Cruickshank G, Teeling JL, *et al.* The intrathecal CD163-
57 751 haptoglobin-hemoglobin scavenging system in subarachnoid

- 1
2
3 752 hemorrhage. *J Neurochem* 2012;**121**:785–92. doi:10.1111/j.1471-
4 753 4159.2012.07716.x
- 5
6 754 24 Kristiansen M, Graversen JH, Jacobsen C, *et al.* Identification of
7 755 the haemoglobin scavenger receptor. *Nature* 2001;**409**:198–201.
8 756 doi:10.1038/35051594
- 9
10 757 25 Bulters D, Gaastra B, Zolnourian A, *et al.* Haemoglobin scavenging
11 758 in intracranial bleeding: biology and clinical implications. *Nat Rev*
12 759 *Neurol* 2018;**14**:416–32. doi:10.1038/s41582-018-0020-0
- 13
14 760 26 Zhao X, Sun G, Ting SM, *et al.* Cleaning up after ICH: The role of
15 761 Nrf2 in modulating microglia function and hematoma clearance. *J*
16 762 *Neurochem* 2015;**133**:144–52. doi:10.1111/jnc.12974
- 17
18 763 27 Morris CM, Candy JM, Edwardson JA, *et al.* Evidence for the
19 764 localization of haemopexin immunoreactivity in neurones in the
20 765 human brain. *Neurosci Lett* 1993;**149**:141–4. doi:10.1016/0304-
21 766 3940(93)90756-B
- 22
23 767 28 Chen M, Regan RF. Time course of increased heme oxygenase
24 768 activity and expression after experimental intracerebral hemorrhage:
25 769 Correlation with oxidative injury. *J Neurochem* 2007;**103**:2015–21.
26 770 doi:10.1111/j.1471-4159.2007.04885.x
- 27
28 771 29 Harada N, Kanayama M, Maruyama A, *et al.* Nrf2 regulates
29 772 ferroportin 1-mediated iron efflux and counteracts
30 773 lipopolysaccharide-induced ferroportin 1 mRNA suppression in
31 774 macrophages. *Arch Biochem Biophys* 2011;**508**:101–9.
32 775 doi:10.1016/j.abb.2011.02.001
- 33
34 776 30 Sandberg M, Patil J, D'Angelo B, *et al.* NRF2-regulation in brain
35 777 health and disease: Implication of cerebral inflammation.
36 778 *Neuropharmacology* 2014;**79**:298–306.
37 779 doi:10.1016/j.neuropharm.2013.11.004
- 38
39 780 31 Pan H, Wang H, Zhu L, *et al.* Depletion of Nrf2 enhances
40 781 inflammation induced by oxyhemoglobin in cultured mice
41 782 astrocytes. *Neurochem Res* 2011;**36**:2434–41. doi:10.1007/s11064-
42 783 011-0571-6
- 43
44 784 32 Chen G, Fang Q, Zhang J, *et al.* Role of the Nrf2-ARE pathway in
45 785 early brain injury after experimental subarachnoid hemorrhage. *J*
46 786 *Neurosci Res* 2011;**89**:515–23. doi:10.1002/jnr.22577
- 47
48 787 33 Li T, Wang H, Ding Y, *et al.* Genetic elimination of Nrf2
49 788 aggravates secondary complications except for vasospasm after
50 789 experimental subarachnoid hemorrhage in mice. *Brain Res*
51 790 2014;**1558**:90–9. doi:10.1016/j.brainres.2014.02.036
- 52
53 791 34 Zhao XD, Zhou YT, Lu XJ. Sulforaphane enhances the activity of
54 792 the Nrf2-ARE pathway and attenuates inflammation in OxyHb-
55 793 induced rat vascular smooth muscle cells. *Inflamm Res*
56 794 2013;**62**:857–63. doi:10.1007/s00011-013-0641-0

- 1
2
3 795 35 Zhao X, Wen L, Dong M, *et al.* Sulforaphane activates the cerebral
4 796 vascular Nrf2–ARE pathway and suppresses inflammation to
5 797 attenuate cerebral vasospasm in rat with subarachnoid hemorrhage.
6 798 *Brain Res* 2016;**1653**:1–7. doi:10.1016/j.brainres.2016.09.035
7
8 799 36 Farrell B, Godwin J, Richards S, *et al.* The United Kingdom
9 800 transient ischaemic attack (UK-TIA) aspirin trial: Final results. *J*
10 801 *Neurol Neurosurg Psychiatry* Published Online First: 1951.
11 802 doi:10.1136/jnnp.54.12.1044
12
13 803 37 Rankin J. Cerebral Vascular Accidents in Patients over the Age of
14 804 60: III. Diagnosis and Treatment. *Scott Med J* Published Online
15 805 First: 1957. doi:10.1177/003693305700200604
16
17 806 38 Quinn TJ, Dawson J, Walters MR, *et al.* Reliability of the modified
18 807 rankin scale: A systematic review. *Stroke* Published Online First:
19 808 2009. doi:10.1161/STROKEAHA.109.557256
20
21 809 39 Jennett B, Bond M. ASSESSMENT OF OUTCOME AFTER
22 810 SEVERE BRAIN DAMAGE. A Practical Scale. *Lancet* Published
23 811 Online First: 1975. doi:10.1016/S0140-6736(75)92830-5
24
25 812 40 Jennett B, Snoek J, Bond MR, *et al.* Disability after severe head
26 813 injury: Observations on the use of the Glasgow Outcome Scale. *J*
27 814 *Neurol Neurosurg Psychiatry* Published Online First: 1981.
28 815 doi:10.1136/jnnp.44.4.285
29
30 816 41 Pace A, Mitchell S, Casselden E, *et al.* A subarachnoid
31 817 haemorrhage-specific outcome tool. *Brain* 2018;**141**:1111–21.
32 818 doi:10.1093/brain/awy003
33
34 819 42 Powell JH, Beckers K, Greenwood RJ. Measuring progress and
35 820 outcome in community rehabilitation after brain injury with a new
36 821 assessment instrument - The BICRO-39 scales. *Arch Phys Med*
37 822 *Rehabil* Published Online First: 1998. doi:10.1016/S0003-
38 823 9993(98)90265-9
39
40 824 43 van Heugten C, Rasquin S, Winkens I, *et al.* Checklist for cognitive
41 825 and emotional consequences following stroke (CLCE-24):
42 826 development, usability and quality of the self-report version. *Clin*
43 827 *Neurol Neurosurg* 2007;**109**:257–62.
44 828 doi:10.1016/j.clineuro.2006.10.002
45
46 829 44 Al-Tamimi YZ, Bhargava D, Feltbower RG, *et al.* Lumbar drainage
47 830 of cerebrospinal fluid after aneurysmal subarachnoid hemorrhage: a
48 831 prospective, randomized, controlled trial (LUMAS). *Stroke*
49 832 2012;**43**:677–82. doi:10.1161/STROKEAHA.111.625731
50
51 833 45 Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to
52 834 subarachnoid hemorrhage visualized by computerized tomographic
53 835 scanning. *Neurosurgery* 1980;**6**:1–
54 836 9.<http://www.ncbi.nlm.nih.gov/pubmed/7354892>
55
56 837 46 Naidech AM, Janjua N, Kreiter KT, *et al.* Predictors and impact of

- 1
2
3 838 aneurysm rebleeding after subarachnoid hemorrhage. *Arch Neurol*
4 839 2005;**62**:410–6. doi:10.1001/archneur.62.3.410
- 5
6 840 47 Zhao J, Kobori N, Aronowski J, *et al.* Sulforaphane reduces infarct
7 841 volume following focal cerebral ischemia in rodents. *Neurosci Lett*
8 842 2006;**393**:108–12. doi:10.1016/j.neulet.2005.09.065
- 9
10 843 48 Zhao X, Sun G, Zhang J, *et al.* Transcription factor Nrf2 protects
11 844 the brain from damage produced by intracerebral hemorrhage.
12 845 *Stroke* 2007;**38**:3280–6. doi:10.1161/STROKEAHA.107.486506
- 13
14 846 49 Alfieri A, Srivastava S, Siow RCM, *et al.* Sulforaphane
15 847 preconditioning of the Nrf2/HO-1 defense pathway protects the
16 848 cerebral vasculature against blood-brain barrier disruption and
17 849 neurological deficits in stroke. *Free Radic Biol Med* 2013;**65**:1012–
18 850 22. doi:10.1016/j.freeradbiomed.2013.08.190
- 19
20 851 50 Sharma V, McNeill JH. To scale or not to scale: The principles of
21 852 dose extrapolation. *Br. J. Pharmacol.* 2009. doi:10.1111/j.1476-
22 853 5381.2009.00267.x
- 23
24 854 51 Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal
25 855 to human studies revisited. *FASEB J* Published Online First: 2007.
26 856 doi:10.1096/fj.07-9574LSF
- 27
28 857 52 Clarke JD, Hsu A, Williams DE, *et al.* Metabolism and tissue
29 858 distribution of sulforaphane in Nrf2 knockout and wild-type mice.
30 859 *Pharm Res* 2011;**28**:3171–9. doi:10.1007/s11095-011-0500-z
- 31
32 860 53 Jazwa A, Rojo AI, Innamorato NG, *et al.* Pharmacological
33 861 Targeting of the Transcription Factor Nrf2 at the Basal Ganglia
34 862 Provides Disease Modifying Therapy for Experimental
35 863 Parkinsonism. *Antioxid Redox Signal* 2011;**14**:2347–60.
36 864 doi:10.1089/ars.2010.3731
- 37
38 865 54 Kumar G, Shahripour RB, Harrigan MR. Vasospasm on transcranial
39 866 Doppler is predictive of delayed cerebral ischemia in aneurysmal
40 867 subarachnoid hemorrhage: a systematic review and meta-analysis.
41 868 2016;**124**:1257–64. doi:10.3171/2015.4.JNS15428.
- 42
43 869 55 Tam AKH, Ilodigwe D, Li Z, *et al.* Global cerebral atrophy after
44 870 subarachnoid hemorrhage: a possible marker of acute brain injury
45 871 and assessment of its impact on outcome. *Acta Neurochir Suppl*
46 872 Published Online First: 2013. doi:10.1007/978-3-7091-1192-5_5
- 47
48 873 56 Nicci Campbell, Carl Verschuur, Sophie Mitchell, Orlaith
49 874 McCaffrey, Lewis Deane, Hannah Taylor, Rory Smith, Lesley
50 875 Foulkes, James Glazier, Angela Darekar, Mark E Haacke, Diederik
51 876 Bulters and IG. Hearing impairment after subarachnoid
52 877 haemorrhage. *Ann Clin Transl Neurol* 2018;**In press**.
53 878 doi:10.1002/acn3.714
- 54
55 879 57 Vergouwen MDI, Vermeulen M, van Gijn J, *et al.* Definition of
56 880 delayed cerebral ischemia after aneurysmal subarachnoid

- 1
2
3 881 hemorrhage as an outcome event in clinical trials and observational
4 882 studies: proposal of a multidisciplinary research group. *Stroke*
5 883 2010;**41**:2391–5. doi:10.1161/STROKEAHA.110.589275
6
7 884 58 Alaraj A, Hussein AE, Esfahani DR, *et al.* Reducing length of stay
8 885 in aneurysmal subarachnoid hemorrhage: A three year institutional
9 886 experience. *J Clin Neurosci* 2017;**42**:66–70.
10 887 doi:10.1016/j.jocn.2017.03.049
11
12 888 59 Teasdale GM, Drake CG, Hunt W, *et al.* A universal subarachnoid
13 889 haemorrhage scale: report of a committee of the world federation of
14 890 Neurosurgical societies. *J. Neurol. Neurosurg. Psychiatry*. 1988.
15 891 doi:10.1136/jnnp.51.11.1457
16
17 892 60 Jaja BNR, Saposnik G, Lingsma HF, *et al.* Development and
18 893 validation of outcome prediction models for aneurysmal
19 894 subarachnoid haemorrhage: The SAHIT multinational cohort study.
20 895 *BMJ* Published Online First: 2018. doi:10.1136/bmj.j5745
21
22 896
23
24
25
26
27
28
29
30
31
32
33
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BMJ Open

A study protocol for SFX-01 After Subarachnoid haemorrhage (SAS): A multi-centre randomised double-blinded, placebo controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-028514.R2
Article Type:	Protocol
Date Submitted by the Author:	26-Dec-2019
Complete List of Authors:	Zolnourian, Ardalan; University of Southampton, Clinical Neuroscience; University Hospital Southampton NHS Foundation Trust, Franklin, Stephen Galea, Ian; University of Southampton Faculty of Medicine, Experimental Neurology Bulters, Diederik ; University Hospital Southampton NHS Foundation Trust
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Medical management, Evidence based practice, Neurology, Surgery, Pharmacology and therapeutics
Keywords:	Randomised controlled trial, Subarachnoid haemorrhage, Nrf2, Sulforaphane, Delayed cerebral ischaemia

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4 1 **A study protocol for SFX-01 After Subarachnoid haemorrhage**
5 2 **(SAS): A multi-centre randomised double-blinded, placebo**
6 3 **controlled trial**
7 4
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9 6

10 6 **Date and version:** 16th May 2018, Version 7
11 7

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50 **Abstract**

51 **Introduction**

54 Subarachnoid haemorrhage from a ruptured cerebral aneurysm carries high morbidity
55 and mortality. Despite huge advances in techniques to secure the aneurysm, there has
56 been little progress in the treatment of the deleterious effects of the haemorrhage.

58 Sulforaphane is an Nrf2 inducer with anti-oxidant and anti-inflammatory properties. It
59 has been shown to improve clinical outcome in experimental models of SAH, but is
60 unstable. SFX-01 (Evgen Pharma) is a novel composition comprised of synthetic
61 sulforaphane stabilised within an α -cyclodextrin complex. On ingestion, the complex
62 releases sulforaphane making SFX-01 an ideal vehicle for delivery of sulforaphane.

64 **Methods and analysis**

66 The objective of the study is to assess the safety, pharmacokinetics and efficacy of
67 SFX-01. This is a prospective, multi-centre, randomised, double-blind placebo-
68 controlled trial in patients aged 18-80 years with aneurysmal subarachnoid
69 haemorrhage in the previous 48 hours. 90 patients will be randomised to receive SFX-
70 01 (300mg) or placebo twice-daily for up to 28 days.

72 Safety will be assessed using blood tests and adverse event reporting.
73 Pharmacokinetics will be assessed based on paired blood and CSF sulforaphane levels
74 on day seven. A subgroup will have hourly samples taken during six hours post-
75 dosing on days one and seven. Pharmacodynamics will be assessed by haptoglobin
76 and malondialdehyde levels, and maximum flow velocity of middle cerebral artery
77 will be measured by transcranial Doppler ultrasound.

79 Clinical outcomes will be assessed at days 28, 90 and 180 with mRS, GOSE, SAHOT,
80 SF-36, BICRO-39 and CLCE-24. MRI at six months including quantitative
81 susceptibility mapping and volumetric T1 will measure iron deposition and cortical
82 volume.

84 Safety, CSF sulforaphane concentration and middle cerebral artery flow velocity will
85 be primary outcomes and all others secondary.

87 **Ethics and dissemination**

88 Ethical approval was obtained from South Central Hampshire A committee.
89 Outcomes of the trial will be submitted for publication in a peer reviewed journal.

90 **Trial registration number:** NCT02614742 ; Pre-results

91

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96 **Strengths and limitations of this study**

- 97 • **A strength of this study is that it tests a new class of drug not previously used**
- 98 **after human SAH.**
- 99 • **It is at low risk of bias due to placebo control and appropriate blinding.**
- 100 • **The study design includes multiple mechanistic outcomes to give deeper**
- 101 **understanding of any clinical findings.**
- 102 • **The study includes multiple novel outcome measures and MRI sequences.**
- 103 **While these may provide new insights, some are exploratory due to limited**
- 104 **experience with them in SAH to date.**
- 105 • **It is a multicentre trial, and its results should be generalizable to patients**
- 106 **with high volume SAH in neurosurgical units in the UK, although due to the**
- 107 **complexity of its design, it has been limited to three centres to ensure high**
- 108 **quality outcome measurement.**

110 **Introduction**

111 Spontaneous Subarachnoid Haemorrhage (SAH) is a devastating cerebrovascular
112 injury with an incidence of 9.1 per 100,000 population [1]. It affects around 7000
113 patients in the UK annually. Around 85% are due to ruptured intracranial aneurysms
114 [2]. The incidence is age related peaking at 52 years. SAH carries a high overall
115 mortality rate of up to 67% [3], and only half of the survivors are able to live
116 independently [4]. It therefore has a high burden on society due to the loss of
117 productivity and resources [5].

118
119 Conventionally following SAH, treatment is primarily directed to securing the
120 aneurysm and prevent further re-bleeding. This however does nothing to ameliorate
121 the morbidity and mortality due to the haemorrhage. The only approved treatment is
122 nimodipine [6]. However, its effects are small and poor outcome remains a significant
123 problem [7]. Moreover, even in survivors considered to have made a good recovery,
124 neurocognitive deficits are common leading to extensive problems with social
125 reintegration and functioning in the workplace [5].

126
127 The mechanism of injury following SAH is multifactorial[8]. Early brain injury (EBI)
128 refers to the processes occurring within the first 72 hours which include blood-brain
129 barrier (BBB) dysfunction [9], cerebral oedema [10][11], neuronal cell death [12],
130 altered ionic homeostasis, excitotoxicity, thrombin activation [13], vascular integrity
131 degradation [14], oxidative stress [15], and inflammation [16]. However, despite the
132 terminology, mechanisms such as oxidative stress and inflammation are not limited to
133 this early period. They continue to worsen beyond the first three days, at the same
134 time as CSF free haemoglobin (Hb) concentration rises markedly as it is released
135 from the clot and mechanisms to dispose of Hb are saturated. It is also in this delayed
136 phase when cerebral vasospasm occurs, affecting both micro- [17] and
137 microvasculature [18].

138
139 Sulforaphane (SFN) is known to upregulate the nuclear factor erythroid 2-related
140 factor 2 (Nrf2) pathway. Nrf2 is a redox-sensitive transcription factor that binds to a
141 specific DNA site, the anti-oxidant response element, upstream of genes encoding
142 detoxifying and anti-oxidant enzymes [19] [20]. Some of these enzymes include

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3 143 glutathione S-transferases (GSTs), NAD(P)H-quinone oxidoreductase 1 (NQO1) and
4 144 haem oxygenase 1 (HO-1) [19]. During physiological conditions Nrf2 is bound to the
5 145 Kelch-like ECH associated protein 1 (KEAP1) in the cytoplasm. In response to stress
6 146 such as SAH, Nrf2 is released from KEAP1 and then translocates to the nucleus
7 147 leading to enhanced gene transcription [21].
8 148

9 149 Nrf2 also upregulates haptoglobin (Hp), an acute phase glycoprotein found in plasma
10 150 [22], as well as cerebrospinal fluid (CSF) [23]. Hp is part of an important Hb
11 151 scavenging pathway after SAH. It binds tightly to free Hb, and this Hb-Hp complex is
12 152 taken up by CD163-positive macrophages [24]. This pathway is saturated after SAH
13 153 [23] and upregulation of Hp represents a possible therapeutic avenue [25]. Nrf2 also
14 154 regulates degradation of red blood cells, and metabolism of haem and iron through
15 155 transcriptional upregulation of CD36 [26], haemopexin [27], HO-1 [28] and ferritin
16 156 [29].
17 157

18 158 Nrf2 is expressed in the central nervous system (CNS) and is upregulated in response
19 159 to inflammation and cerebral insults [30]. Nrf2 knockout is associated with a more
20 160 pronounced inflammatory response *in vitro* [31] and *in vivo* [32], and the increased
21 161 inflammatory response is associated with more brain oedema, cell death and poorer
22 162 neurological recovery [33]. SFN increases HO-1, NQO1, and GST- α 1 levels and
23 163 reduces IL-1 β , IL-6, and TNF- α [34]. It leads to a reduction in vasospasm and
24 164 improves neurological recovery [32] [35].
25 165

26 166 SFN has a relatively short half-life rendering it impractical for clinical use. SFX-01
27 167 (Evgen Pharma) is a novel new agent comprising SFN complexed with α -cyclodextrin
28 168 which is suitable for clinical use. On ingestion, SFN is released from the α -
29 169 cyclodextrin and is an effective method to deliver SFN. While cyclodextrin catalyses
30 170 the reaction with the intermediate that is used to create the sulforaphane, it serves a
31 171 very important purpose of creating a 'scaffold' around sulforaphane, increasing its
32 172 shelf-life and half-life. In two Phase I trials (NCT01948362, NCT02055716) no
33 173 serious adverse events were reported in healthy volunteers. Here we describe the
34 174 protocol for a phase II trial of SFX-01 in patients who have suffered a SAH.
35 175

36 176 **Objectives**

37 177
38 178 The objective of the study is to assess the safety, pharmacokinetics and efficacy of
39 179 SFX-01.
40 180

41 181 **Methods**

42 182 ***Trial design***

43 183
44 184
45 185 This is a prospective, double-blind, parallel group, randomised controlled trial
46 186 comparing SFX-01 (300 mg) taken orally as capsules or as a suspension via a
47 187 nasogastric tube (NG) twice-daily for up to 28 days *versus* placebo in patients with
48 188 SAH within 48 hours of enrolment.
49 189

50 190 The treatment window of 48 hours was selected as the best compromise between
51 191 competing factors. SFX-01 has pleiotropic actions against multiple mechanisms each

1
2
3 192 with different temporal profiles. Since SAH is an acute unpredictable event, the
4 193 earliest one would be able to start treatment is soon after ictus, on admission into
5 194 hospital. This would give optimal protection against early brain injury. Initiating
6 195 treatment would still be justified up to 72 hours post-ictus, after which delayed
7 196 cerebral ischaemia (DCI) occurs, such that a delay of more than 72 hours would be
8 197 expected to undermine treatment efficacy. Within the initial 72 hours, earlier
9 198 treatment would allow more time for Nrf2 pathway activation and expression of its
10 199 transcriptome to protect against delayed events, as well as provide more opportunity
11 200 for SFX-01 to act against early brain injury. On the other hand the earlier one
12 201 stipulates treatment would start, the more patients one will exclude from the study. No
13 202 animal studies have been conducted to investigate the timing of sulforaphane
14 203 administration, and even if available, extrapolation of timing from animal models has
15 204 its limitations due to the much quicker clot resorption in rodents. After considering all
16 205 these factors, it was decided to start SFX-01 treatment at the earliest possible
17 206 opportunity after SAH, yet still allow patients to be included if their presentation was
18 207 delayed to some extent, to ensure generalizability to real clinical practice where
19 208 delays in admission to tertiary centres are not uncommon. An audit of the lead study
20 209 centre showed that most patients are admitted within 48 hours, leading to the adoption
21 210 of SAH within 48 hours as the inclusion criterion, striking the best compromise
22 211 between the practicality of recruitment and the need to start treatment early.
23 212

24 213 Detailed pharmacovigilance will inform the safety of SFX-01. Pharmacokinetics will
25 214 be determined by measuring levels of SFN and its metabolites in CSF and blood at
26 215 day seven in all patients; a more detailed profile will be obtained in a subgroup of up
27 216 to 12 patients who will have hourly blood and CSF samples for six hours after dosing
28 217 on days one and seven. Pharmacodynamics will be assessed with blood Hp and
29 218 malondialdehyde (MDA) levels, and middle cerebral artery (MCA) flow velocity as
30 219 measured on transcranial Doppler (TCD) ultrasound as an estimate of large cerebral
31 220 artery spasm. Longer term outcome will be assessed using validated outcome scales,
32 221 at day 28 using Modified Rankin Scale (mRS) ([36] [37] [38] , Glasgow Outcome
33 222 Score (GOSE) [39][40] and the SAH Outcome Tool (SAHOT) [41] as well as at day
34 223 90 and 180 using the mRS, GOSE, SAHOT, Brain Injury Community Rehabilitation
35 224 Outcome Scales (BICRO-39) [42] and the Check List for Cognitive and Emotional
36 225 consequences following stroke (CLCE-24) [43].
37 226

38 227 *Patient and public involvement*

39 228

40 229 The overall design of the study including planned investigations i.e. lumbar puncture
41 230 (LP), magnetic resonance imaging (MRI), blood tests, outcome questionnaires, the
42 231 treatment methods and the consent procedures were discussed in a local SAH support
43 232 group consisting of individuals with a previous history of SAH as well as their
44 233 relatives. The meeting was led by the chief investigator, a consultant neurovascular
45 234 surgeon, and the neurovascular specialist nurse who are normally the main point of
46 235 contact for SAH patients. The results were used to inform planning of the trial.
47 236 Particular attention was given to the study lumbar puncture which was felt to be
48 237 justified on the grounds that the majority of patients will undergo CSF diversion for
49 238 clinical reasons anyway, and evidence from a randomised controlled trial that CSF
50 239 diversion in all Fisher 3&4 patients causes no harm and appears to provide short term
51 240 symptomatic benefit [44]. The meeting was also beneficial in shaping and improving
52 241 the patient information sheet and consent forms.

1
2
3 2424 243 ***Study setting***

5 244

7 245 This is a multicentre study conducted in regional neurosciences centres with specialist
8 246 services to treat aneurysmal SAH. Patients will be identified at referral to the
9 247 admitting neurosurgical or neurointensive care units. After informed consent, dosing
10 248 and study interventions will occur in the neurosciences centre until patients are
11 249 discharged to either home or their local hospitals. There are three recruiting centres;
12 250 University Hospital Southampton, Royal Infirmary of Edinburgh and the Royal
13 251 London Hospital. Upon discharge, follow-up may take place in clinic, or at visits to
14 252 district general hospitals and rehabilitation centres, or patients' residences.
15 253

16 253

17 254 **Eligibility Criteria**

18 255

19 256 ***Inclusion criteria***

20 257

- 22 258 1. Patients with radiological evidence of spontaneous aneurysmal SAH
- 23 259 2. Fisher grade 3 or 4 on CT
- 24 260 3. Definitive treatment of aneurysm has not been ruled out
- 25 261 4. Previously living independently
- 26 262 5. In the opinion of the investigator, the delay from ictus to randomisation and
27 263 initiation of trial medication will not exceed 48 hours
- 28 264 6. Aged 18 to 80 years
- 29 265 7. In the opinion of the investigator it will be possible to obtain Informed Consent
30 266 from the Patient, Personal Legal Representative or Professional Legal
31 267 representative within 24 hours of first dose

32 268

33 269 ***Exclusion criteria***

34 270

- 35 271 1. Traumatic SAH
- 36 272 2. Fisher grade 1 or 2
- 37 273 3. SAH diagnosed on LP with no evidence of blood on CT
- 38 274 4. Decision not to treat aneurysm has been made
- 39 275 5. Plan to withdraw treatment
- 40 276 6. Significant kidney disease as defined as plasma creatinine ≥ 2.5 mg/dL (221
41 277 $\mu\text{mol/l}$)
- 42 278 7. Liver disease as defined as total bilirubin ≥ 2 -fold the upper limit of normal, as
43 279 measured by the local laboratory
- 44 280 8. Females who are pregnant or lactating
- 45 281 9. Participants enrolled in another interventional research trial in the last 30 days
- 46 282 10. Patients for whom it is known, at the time of screening, that clinical follow-up will
47 283 not be feasible
- 48 284 11. Patients unwilling to use two forms of contraception (one of which being a barrier
49 285 method) for 90 days (men) or 30 days (women) after the last trial medication dose
- 50 286 12. Known hypersensitivity to any component of a SFN containing product including
51 287 broccoli

52 288

53 289 Recruitment will be limited to Fisher grade 3 and 4 SAH. These patients represent the
54 290 majority of aneurysmal SAH. They have a higher volume of haemorrhage with a

291 poorer outcome and more delayed neurological deficits [45]. They are therefore
292 mechanistically and clinically expected to derive greatest benefit from SFX-01. In
293 addition to using Fisher scale, all baseline CT scans will undergo volumetric analysis
294 of blood load, which are more objective and reliable than any versions of the Fisher
295 scale.

296
297 Inclusion of patients with unsecured aneurysms would risk a high incidence of
298 rebleeding in the study. Rebleeding is associated with exceedingly bad outcomes [46],
299 masking any effect from SFX-01. However, since not all aneurysms are secured
300 within 48 hours, there will be no requirement for the aneurysm to have been secured
301 prior to enrolment. Instead patients in whom treatment of the aneurysm has been ruled
302 out due to poor clinical status will be excluded.

303
304 Although there are no known risks to kidney or liver, due to the relative inexperience
305 with SFX-01 in humans, patients with liver or kidney problems will be excluded.

306 307 **Intervention**

308 309 ***Trial medication***

310
311 SFX-01 (active 300 mg capsule) or placebo (α -cyclodextrin only capsule) will be
312 taken orally or as a suspension via a nasogastric tube (NG) twice daily for up to 28
313 days. Pharmagra manufacture the active pharmaceutical ingredient (API) in North
314 Carolina, US. The API is then encapsulated at Quotient in Reading, UK. The API
315 manufacture has been audited by the FDA and Evgen pharma. SFX-01 and placebo
316 capsules as well as their contents will be identical in appearance. They will be stored
317 at 2-8 degrees Celsius.

318
319 Animal studies in ischaemic stroke, intracerebral haemorrhage and SAH have all used
320 5 mg/kg dose of SFN in rodents [32] [47] [48] [49]. Conversion of animal doses to
321 humans using body surface area, as has been widely recommended [50] [51], yields a
322 human dose of 50 mg SFN. This is equivalent to 300 mg of SFX-01 containing 46.15
323 mg of SFN. In the clinical studies conducted to date, SFX-01 has been shown to be
324 well tolerated at doses of 600mg once daily and 300mg twice daily with no serious
325 adverse effects. Therefore no further dose ranging studies were performed.

326 327 ***De-escalation from the trial regimen***

328
329 In the event of tolerability problems whilst the patient is in the neurosurgical centre,
330 the Investigator will assess whether simple measures to ease the effects of the adverse
331 event(s) may be implemented (i.e. antacid in the case of GI irritation or anti-emetic in
332 the event of nausea).

333 The investigator will also assess whether or not the adverse event(s) could be related
334 to the trial medication and severe enough to warrant a dose frequency reduction. In
335 the first instance the investigator may consider missing one dose. If a dose frequency
336 reduction is warranted, from that point onwards the second dose of the day will be
337 omitted; a dose frequency increase back to twice daily will not be permitted.

338
339 If tolerability problems continue then the investigational medication will be stopped;
340 patients will continue in the study and complete the study visits. The staged dose

1
2
3 341 frequency de-escalation (dropping to once daily) will not be carried out after
4 342 discharge from the neurosurgical centre; if tolerability problems occur after discharge,
5 343 medication will be stopped; patients will continue in the study and complete the study
6 344 visits in accordance with the schedule of assessments.
7
8 345

9 346 *Treatment compliance*

10 347
11 348 Compliance with treatment will be recorded during the inpatient hospital stay by
12 349 health care professionals and/or a member of the research team. On discharge to the
13 350 usual residence, responsibility for this will be transferred to the patient or their
14 351 Personal Legal Representative, aided by detailed instructions. In the event of
15 352 discharge to a rehabilitation unit or patient local hospital, written instructions will be
16 353 provided on discharge and verbal communication with the clinical team will ensure
17 354 compliance.
18 355

19
20 356 All patients will be discharged with a patient diary which will be filled in and
21 357 collected at day 28. Compliance will be further monitored by drug reconciliation.
22 358 Patients will be asked to return the medication bottle and any residual contents at the
23 359 day 28 visit. At this time any residual tablets will be counted and recorded.
24 360

25 361 *Concomitant treatment*

26 362
27 363 There are no known drug interactions, and participation in the trial will not alter
28 364 routine treatment of SAH. Participation in other interventional research studies will
29 365 not be allowed until after the last follow up visit.
30 366

31 367 Outcomes

32 368 Primary end-points

33 369 *Safety*

34 370
35 371
36 372
37 373 To evaluate the safety of up to 28 days of SFX-01 dosed at up to an equivalent of
38 374 92mg SFN per day.
39 375

40 376 Prior to the start of this study, the trial medication had only been used in healthy
41 377 individuals and not in a patient population. Therefore safety will be one of the main
42 378 objectives of this study. This will be evaluated through routine tests (full blood count,
43 379 urea and electrolytes, coagulation screen, liver function tests, and urine microscopy)
44 380 at baseline, post-dose, day seven and day 28 as well as close monitoring of patients
45 381 for any side-effects or adverse events. Adverse events will be coded following
46 382 MEDDRA and followed up until resolution and graded for severity. Incidence of dose
47 383 de-escalation or discontinuation will also be reported.
48 384

49 385 *Pharmacokinetic*

50 386
51 387 To detect the presence of SFN and its metabolites in CSF and blood.
52 388

53 389 Animal models have shown that SFN crosses the blood-brain barrier and can therefore
54 390 be detected in the brain [52] [53] There is some variation in the levels achieved in

391 these studies, and it has not been studied in humans. All patients will have a paired
392 CSF/blood sample taken at seven days post-ictus. This will be via a LP unless the
393 patient has an external ventricular drain (EVD) for their clinical care in which case it
394 will be obtained from the EVD. In addition, up to 12 patients with an EVD will be
395 asked to consent to hourly CSF and blood samples for six hours after dosing on days
396 one and seven. Hourly CSF sampling will be performed by trained study personnel
397 using a bespoke sterile closed cascade of syringes so that the EVD line is accessed
398 directly only once to reduce the risk of infection. Sample collection and processing
399 are detailed in specific study operating procedures.

401 *Vasospasm*

402
403 To determine if a minimum of seven days treatment with SFX-01 reduces MCA peak
404 flow velocity following SAH.

405
406 TCD ultrasound will be used to measure the MCA, ICA and ECA maximum flow
407 velocity and the Lindegaard ratio will be calculated. TCDs will be performed on
408 alternate daily basis including at baseline. They will be performed for at least seven
409 days or until no longer clinically indicated. Blood flow velocity is measured in
410 cm/second and is inversely related to the luminal diameter of the vessel. The greater
411 this value the more likely the degree of narrowing or spasm in the vessel. It has a
412 good predictive value for DCI. A recent systematic review and meta-analysis, which
413 pooled data from 2870 patients, showed a sensitivity of 90%, specificity of 71%, and
414 negative and positive predictive values of 92% and 57% respectively [54].

416 Secondary end-points

418 *Pharmacodynamic*

420 *CSF and blood – Haptoglobin and Malondialdehyde*

421
422 Hp represents a major Hb detoxification pathway and is upregulated by Nrf2. MDA is
423 a measure of oxidative stress. Hp and MDA will be measured in blood at baseline,
424 day seven and day 28, and in CSF on day seven. A local audit at the main study
425 neurosurgical centre showed that approximately 1/3 of patients have an EVD sited as
426 part of their routine clinical care to treat hydrocephalus. In these patients additional
427 samples will be obtained. This will allow investigation of the temporal profile of Hp
428 level and oxidative stress. Additionally, there will be exploratory investigations using
429 proteomics, transcriptomics and genomics using CSF and blood samples.

431 *MRI – iron and brain volume*

432
433 All patients will have MRI 180 days after SAH. Brain volume on T1 sequences will
434 be measured, since this has been shown to correlate with outcome [55]. Cortical iron
435 content will be assessed using quantitative susceptibility mapping after susceptibility
436 weighted MR imaging, which predominantly measures siderotic iron deposits [56].
437 Iron is a major component of Hb, and it is unknown what effects SFX-01, SFN or
438 increased Hp binding of Hb may have on the downstream iron pathway.

440 *Clinical outcome*

- 1
2
3 441
4 442 • mRS at seven days, discharge, 28, 90 and 180 days.
5 443 • Incidence of DCI defined as a new focal deficit or reduction in GCS (by two
6 444 points or more) if not explained by other causes (i.e. re-bleed, hydrocephalus,
7 445 seizure, meningitis, sepsis or hyponatraemia) [57]
8 446 • Incidence of new cerebral infarct on CT or MRI
9 447 • Institution of hypertensive therapy for presumed DCI
10 448 • SF-36 quality of life survey at 28, 90 & 180 days
11 449 • CLCE-24 and BICRO-39 at 90 & 180 days
12 450 • SAHOT and GOSE at 28, 90 & 180 days
13 451 • Length of acute hospital stay
14 452 • Discharge destination
15 453

16 454 A number of measures of efficacy have been selected reflecting the most common
17 455 stroke outcome assessment (mRS), the most common brain injury assessment
18 456 (GOSE), the most common quality of life survey (SF-36) and the only SAH specific
19 457 outcome tool [41] to determine the most sensitive tool and make estimates of effect
20 458 size. SAHOT includes 56 items dealing with cognitive, physical, and
21 459 behavioural/psychological consequences of SAH, developed in a SAH focus group by
22 460 patients and experts in the field. This outcome tool has been validated and proposed
23 461 as a more sensitive and responsive tool in SAH [41]. All these tools will be
24 462 administered by research nurses or doctors trained in mRS and GOSE in person, or in
25 463 the event this is not feasible, by phone.
26 464

27 465 A number of short term patient outcomes related to the incidence of DCI are included
28 466 *a priori* such as incidence of infarction as adjudicated by a blinded consultant
29 467 neuroradiologist (with baseline CT and follow up MRI), and institution of
30 468 hypertensive therapy which is defined as institution of inotropes to increase blood
31 469 pressure in intensive care. Several simple outcomes including length of stay and
32 470 discharge destination that have been associated with outcome [58] are also included *a*
33 471 *priori* in the event any patients are lost to longer term follow up.
34 472

35 473 **A summary of all the key study activities with their specific time**
36 474 **points are outlined in table 1.**

37 475
38 476 **Table 1. Schedule of assessments**
39 477

Time points ¹	D 0-2	D 1-3 (12-24 post- dose)	Ongoing assessment (Alternate days) ⁶ (+/-1)	D 7 (+/-1)	Discharge (-2)	D 28 (-6/+2)	D 90 (+/-14)	D 180 (+/- 28)
Study procedures								
Consent	X							
Inclusion/exclusion	X							
WFNS grading	X							
IMP treatment	X							
Safety bloods ²	X	X	X	X	X	X		
Safety urine	X			X	X	X		
Lipid profile	X			X	X	X		
Coagulation screen								

TCD readings ³		X	X	X				
HP, MDA (Blood/CSF) ⁴	X			X		X		
SFN & metabolites (Blood/CSF) ⁵				X				
Pregnancy test	X							
mRS				X	X	X	X	X
GOSE						X	X	X
SAHOT						X	X	X
SF-36						X	X	X
BICRO-39							X	X
CLCE-24							X	X
MRI								X

- 1- Ictus is defined as the onset of symptoms/haemorrhage and is referred to as day 0.
- 2- Safety bloods include: Biochemistry: Sodium, Potassium, Urea, Creatinine, Glucose, Calcium, Total Bilirubin, Alkaline Phosphatase, Alanine Transaminase, Albumin, C-Reactive Protein, and Haematology including Haemoglobin, White Blood Cell Count, Neutrophils (Absolute), Lymphocytes (Absolute), Platelets. These will be done at least on alternate days until no longer clinically indicated.
- 3- TCDs will be performed at baseline before day 3 and will be repeated on alternate daily basis until at least day 7 or where clinically indicated.
- 4- Hp and MDA will be assayed in both CSF and blood at baseline, where possible i.e if patient has an EVD fitted this will be measured in the CSF and blood at baseline as well as every other day until EVD is removed. All patients will have pP and MDA assayed on either a LP or EVD sample on day 7.
- 5- SFN and its metabolites will be measured on day 7 in all patient with paired blood and CSF (LP or EVD sample).
- 6- These assessments will be done on every other day basis with a +/- 1 day window. They will be carried on until discharge or up to when it is clinically required.

Sample Size

No formal sample size calculation has been carried out; the power associated with a sample size of 90 is based on the following assumptions:

- The error probability for the Type I error should not exceed 5% for a one-sided test;
- The primary endpoint will be compared between treatment groups by means of a t-test
- The mean maximum MCA flow velocity for patients treated with SFX-01 is estimated as 175 cm/s and
- The standard deviation of maximum MCA flow velocity is 50 cm/s

Under these assumptions 90 patients will give 80% power to detect a difference in maximum MCA velocity which is approximately half of the standard deviation of the mean value. The standard deviation was assumed to be approximately 30% of the mean value.

Recruitment

Patients with SAH who present to the clinical centres and meet the above criteria will be considered for recruitment. The inclusion and exclusion criteria reflect national practice. Patients will be identified by the treating clinical team at the time of referral, admission or daily medical handover.

518 **Assignment of intervention**

519

520 ***Randomisation and blinding***

521

522 Patients will be randomised in a 1:1 ratio to the active or placebo arm. Randomisation
523 will be stratified using the most recent World Federation of Neurosurgical Societies
524 (WFNS) grade [59] prior to randomization. Patients in different WFNS groups will
525 have significantly different outcomes [60] and imbalance between treatment arms
526 risks treatment allocation bias.

527

528 All treatment packs will be otherwise identical in appearance. Placebo capsules will
529 be identical and contain α -cyclodextrin making the contents indistinguishable should
530 they be opened either inadvertently or for the purposes of NG administration. Patients
531 will be randomised to one of the treatment groups by allocation of the appropriate,
532 sequentially numbered treatment pack from either the clinical trials pharmacy, or a
533 suitably calibrated and monitored fridge outside the pharmacy. The treatment packs
534 will be pre-numbered according to a block balanced randomisation code with a ratio
535 of 1:1 by a blinded third party. They will be selected as per WFNS grading by a
536 member of a research team from pharmacy.

537

538 ***Unblinding***

539

540 The Pharmacy will receive a sealed envelope containing the identity of each trial
541 medication bottle. An envelope may be opened only in the case of a serious adverse
542 event and only when it is essential to the subsequent management of the patient. The
543 decision to unblind will be made in discussion between the treating clinician and PI
544 and where possible CI. The independent trial centre will be responsible for breaking
545 codes for regulatory submissions of Suspected Unexpected Serious Adverse
546 Reactions (SUSARs), thereby maintaining the overall confidentiality of the code
547 breaks. If the code is broken the data for that patient will be excluded from the Per
548 Protocol Population analysis but included in the Intention to Treat Analysis. They will
549 continue in the study and complete the study visits in accordance with the study visit
550 schedule.

551

552 **Data collection and management**

553

554 Data collection will be performed by Good Clinical Practice-trained members of the
555 research team. Study specific training and additional training in disease specific
556 questionnaires will be provided. The data will be entered into a secure electronic case
557 report form.

558

559 ***Statistical analysis***

560

561 The following populations will be considered for the analysis:

- 562 • Intention-to-Treat population (ITT): all randomised patients who receive at
563 least one dose of study medication and with any post-dose efficacy
564 evaluations. Patients where the time from ictus to admission is unknown are
565 to be considered as part of the ITT population .

- 1
2
3 566
- 4 567 • Per-protocol population (PPP): The Per Protocol Population (for Primary
 - 5 568 analysis) will be considered to be those patients in the ITT population that
 - 6 569 have been dosed for a minimum to day seven post ictus without any major
 - 7 570 protocol violations (i.e. wrong inclusions, etc.).
 - 8 571 • Safety population: all randomised patients who have taken at least one dose of
 - 9 572 study medication.

10 573 Last observation carried forwards (LOCF) will be used to impute missing outcome at
11 574 6 months. The final full statistical analysis plan will be published prior to unblinding.

12 575 13 576 **Monitoring**

14 577
15 578 A data safety monitoring board (DSMB) has been set-up to monitor the safety aspect
16 579 of the trial throughout. The DSMB will be independent of the study team and
17 580 company and has its own charter. A steering committee (consisting of the Chief
18 581 Investigator and the sponsor's Chief Medical Officer) will receive and review the
19 582 reports from DSMB, and take action as appropriate.

20 583
21 584 The first 20 patients will only be dosed as an inpatient and may therefore have courses
22 585 shorter than 28 days. The DSMB plan to hold an initial meeting after recruitment of
23 586 20 patients who will have completed seven days of trial medication. If there are no
24 587 safety concerns, further patients will be allowed to be dosed with the trial drug after
25 588 discharge to other hospitals or home. The DSMB will also meet if there are any
26 589 SUSARs or if two patients have had an increase in the grading of the severity of
27 590 adverse events.

28 591
29 592 Recruitment will stop once the target has been reached or if DSMB deems the study
30 593 or trial drug to be associated with a significant number of adverse events compared to
31 594 the normal patient population. The recruitment target is set to a minimum of 90
32 595 patients between three centres in the United Kingdom. Up to 120 patients may be
33 596 recruited in order to allow for withdrawals and deaths.

34 597
35 598 External monitoring will occur regularly throughout the study and after the study has
36 599 been completed. At these visits the monitor(s) will inspect various study records; case
37 600 report forms, investigator site file and source data, provided that subject
38 601 confidentiality is respected.

39 602 40 603 **Adverse events reporting**

41 604
42 605 All adverse events, adverse drug reactions and serious adverse events (SAE) will be
43 606 accurately documented. The AEs are graded in both severity and seriousness i.e mild,
44 607 moderate and severe. Where severe on the severity scale subsequent SAEs will be
45 608 completed instead, within 24 hours of the research team being informed. At each visit
46 609 all AEs will be reassessed to ensure no change has occurred from the previous
47 610 assessment. Monitoring and auditing is done against the original documentation on a
48 611 six-weekly basis by the external monitor. All AEs are also entered onto an electronic
49 612 case report form where further external monitoring will be done.

50 613
51 614 Pregnancies occurring during the study must be reported immediately. In the event it
52 615 does occur, patients will be referred for close obstetric monitoring. All obstetric visits

1
2
3 616 will be monitored closely by the research team and any concerns will be highlighted
4 617 and addressed accordingly.
5 618
6 619

620 ***Data Availability***

621
622 Upon completion of the study, data will be shared with other eligible investigators
623 through academically established means. The datasets used and/or analysed during the
624 study will be available from the corresponding author on reasonable request.
625

626 **Ethical considerations and Informed Consent**

627 ***Consent procedures and emergency dosing***

628
629
630 Most patients with acute SAH present with either severe headache or altered level of
631 consciousness. Many will lack capacity with no legal representative immediately
632 available. SAH is an acute emergency and any benefit from SFX-01 is likely to be
633 greatest the earlier it is administered. The study has therefore been granted ethical
634 permission to obtain baseline blood testing and administer two doses of trial drug
635 without consent if the patient is lacking in capacity and no legal representative is
636 available. If no consent can be obtained at that point, the patient will be withdrawn
637 from the study.
638
639

640 Consent will be obtained in one of three scenarios:

- 641 1- Patients with capacity
- 642 2- Patients without capacity, but with a relative or next of kin available
643 immediately in person
- 644 3- Patients without capacity and no relative or next of kin immediately available
645 in person. In this case, a professional legal representative will be approached.
646

647 ***Re-Consent***

648
649
650 This must take place in two different scenarios:

- 651 1- When patients regain capacity, they must be re-consented.
- 652 2- When patients have been consented through a professional legal
653 representative, after which time either they regaine capacity or the next of kin
654 becomes available.
655

656 **Discussion**

657
658 There is mounting evidence supporting the role of the Nrf2 pathway in outcome after
659 SAH. SFN upregulates Nrf2 expression and improves outcome in animal models.
660 SFX-01 represents an exciting and novel way to deliver SFN to SAH patients with the
661 potential to improve their lives.
662

663 This trial will investigate the safety, pharmacokinetics and pharmacodynamics of
664 SFX-01 after SAH. If successful it may deliver long-term benefit to patients who have

665 suffered SAH and provide new hope to a group of patients characterised by complex
666 neurocognitive problems and disability.

667

668 **Footnotes**

669 **Authors' contributions:**

670 DB conceived the trial. AZ, IG and DB were all involved in the design of the study
671 and its setup. AZ and DB wrote the study protocol. DB managed the recruitment of
672 other centres. SF, IG and DB reviewed the protocol manuscript and approved the final
673 version.

674

675 **Acknowledgements:**

676 The protocol was revised and finalised in conjunction with David Howatt and Robert
677 Holland previously at Evgen Pharma. The manuscript was reviewed by Sally Ross
678 and Thomas Morris of Evgen Pharma.

679

680 **Funding:**

681 This study is funded and sponsored by EvgenPharma.

682

683 **Competing interests:**

684 Dr Stephen Franklin is the Chief Executive Officer of Evgen Pharma plc. All the
685 other authors have no financial or non-financial interest in Evgen Pharma plc.

686

687 **Ethical approval:** South Central Research Ethics Committee, Hampshire A, UK.

688 **Word count:** 5220

689

690 **References**

691

- 692 1 de Rooij NK, Linn FHH, van der Plas JA, *et al.* Incidence of
693 subarachnoid haemorrhage: a systematic review with emphasis on
694 region, age, gender and time trends. *J Neurol Neurosurg Psychiatry*
695 2007;**78**:1365–72. doi:10.1136/jnnp.2007.117655
- 696 2 Van Gijn J, Rinkel GJE. Subarachnoid haemorrhage: Diagnosis,
697 causes and management. *Brain*. 2001. doi:10.1093/brain/124.2.249
- 698 3 Nieuwkamp DJ, Setz LE, Algra A, *et al.* Changes in case fatality of
699 aneurysmal subarachnoid haemorrhage over time, according to age,
700 sex, and region: a meta-analysis. *Lancet Neurol* 2009;**8**:635–42.
701 doi:10.1016/S1474-4422(09)70126-7
- 702 4 Kirkpatrick P, Lindsay K, Shaw M, *et al.* *National Study of*
703 *Subarachnoid Haemorrhage*. 2006.
- 704 5 Rivero-Arias O, Gray A, Wolstenholme J. Burden of disease and
705 costs of aneurysmal subarachnoid haemorrhage (aSAH) in the
706 United Kingdom. *Cost Eff Resour Alloc* 2010;**8**:1–12.
707 doi:10.1186/1478-7547-8-6
- 708 6 Pickard JD, Murray GD, Illingworth R, *et al.* Effect of oral
709 nimodipine on cerebral infarction and outcome after subarachnoid
710 haemorrhage : British aneurysm nimodipine trial. *Bmj*

- 1
2
3 711 1989;**298**:636–42. doi:10.1136/bmj.298.6674.636
- 4 712 7 Dorhout Mees SM, Rinkel GJ, Feigin VL, *et al.* Calcium
5 713 antagonists for aneurysmal subarachnoid haemorrhage. *Cochrane*
6 714 *Database Syst Rev* Published Online First: 2007.
7 715 doi:10.1002/14651858.CD000277.pub3
- 8 716 8 Zolnourian A, Galea I, Bulters D. Neuroprotective Role of the Nrf2
9 717 Pathway in Subarachnoid Haemorrhage and Its Therapeutic
10 718 Potential. *Oxid Med Cell Longev* 2019;**2019**:1–21.
11 719 doi:10.1155/2019/6218239
- 12 720 9 Dóczy T. The pathogenetic and prognostic significance of blood-
13 721 brain barrier damage at the acute stage of aneurysmal subarachnoid
14 722 haemorrhage. Clinical and experimental studies. *Acta Neurochir*
15 723 *(Wien)* 1985;**77**:110–
16 724 32.<http://www.ncbi.nlm.nih.gov/pubmed/4072781>
- 17 725 10 László FA, Varga C, Dóczy T. Cerebral oedema after subarachnoid
18 726 haemorrhage. Pathogenetic significance of vasopressin. *Acta*
19 727 *Neurochir (Wien)* 1995;**133**:122–
20 728 33.<http://www.ncbi.nlm.nih.gov/pubmed/8748754>
- 21 729 11 Zetterling M, Hallberg L, Ronne-Engström E. Early global brain
22 730 oedema in relation to clinical admission parameters and outcome in
23 731 patients with aneurysmal subarachnoid haemorrhage. *Acta*
24 732 *Neurochir (Wien)* 2010;**152**:1527–33. doi:10.1007/s00701-010-
25 733 0684-8
- 26 734 12 Cahill WJ, Calvert JH, Zhang JH. Mechanisms of early brain injury
27 735 after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*
28 736 2006;**26**:1341–53. doi:10.1038/sj.jcbfm.9600283
- 29 737 13 Lad SP, Hegen H, Gupta G, *et al.* Proteomic biomarker discovery in
30 738 cerebrospinal fluid for cerebral vasospasm following subarachnoid
31 739 hemorrhage. *J Stroke Cerebrovasc Dis* 2012;**21**:30–41.
32 740 doi:10.1016/j.jstrokecerebrovasdis.2010.04.004
- 33 741 14 Kusaka G, Ishikawa M, Nanda A, *et al.* Signaling pathways for
34 742 early brain injury after subarachnoid hemorrhage. *J Cereb Blood*
35 743 *Flow Metab* Published Online First: 2004.
36 744 doi:10.1097/01.WCB.0000125886.48838.7E
- 37 745 15 Gaetani P, Pasqualin A, Rodriguez y Baena R, *et al.* Oxidative
38 746 stress in the human brain after subarachnoid hemorrhage. *J*
39 747 *Neurosurg* Published Online First: 2009.
40 748 doi:10.3171/jns.1998.89.5.0748
- 41 749 16 Fassbender K, Hodapp B, Rossol S, *et al.* Inflammatory cytokines
42 750 in subarachnoid haemorrhage: Association with abnormal blood
43 751 flow velocities in basal cerebral arteries. *J Neurol Neurosurg*
44 752 *Psychiatry* Published Online First: 2001. doi:10.1136/jnnp.70.4.534
- 45 753 17 Pennings FA, Bouma GJ, Ince C. Direct observation of the human

- 1
2
3 754 cerebral microcirculation during aneurysm surgery reveals
4 755 increased arteriolar contractility. *Stroke* 2004;**35**:1284–8.
5 756 doi:10.1161/01.STR.0000126039.91400.cb
6
7 757 18 Dorsch NWC, King MT. A review of cerebral vasospasm in
8 758 aneurysmal subarachnoid haemorrhage Part I: Incidence and effects.
9 759 *J. Clin. Neurosci.* 1994. doi:10.1016/0967-5868(94)90005-1
10 760 19 Itoh K, Chiba T, Takahashi S, *et al.* An Nrf2/small Maf heterodimer
11 761 mediates the induction of phase II detoxifying enzyme genes
12 762 through antioxidant response elements. *Biochem Biophys Res*
13 763 *Commun* 1997;**236**:313–22. doi:10.1006/bbrc.1997.6943
14 764 20 Venugopal R, Jaiswal a K. Nrf2 and Nrf1 in association with Jun
15 765 proteins regulate antioxidant response element-mediated expression
16 766 and coordinated induction of genes encoding detoxifying enzymes.
17 767 *Oncogene* 1998;**17**:3145–56. doi:10.1038/sj.onc.1202237
18 768 21 Itoh K, Ishii T, Wakabayashi N, *et al.* Regulatory mechanisms of
19 769 cellular response to oxidative stress. *Free Radic Res* 1999;**31**:319–
20 770 24. doi:10.1080/10715769900300881
21 771 22 Zhao X, Song S, Sun G, *et al.* Neuroprotective Role of Haptoglobin
22 772 after Intracerebral Hemorrhage. *J Neurosci* 2009;**29**:15819–27.
23 773 doi:10.1523/JNEUROSCI.3776-09.2009
24 774 23 Galea J, Cruickshank G, Teeling JL, *et al.* The intrathecal CD163-
25 775 haptoglobin-hemoglobin scavenging system in subarachnoid
26 776 hemorrhage. *J Neurochem* 2012;**121**:785–92. doi:10.1111/j.1471-
27 777 4159.2012.07716.x
28 778 24 Kristiansen M, Graversen JH, Jacobsen C, *et al.* Identification of
29 779 the haemoglobin scavenger receptor. *Nature* 2001;**409**:198–201.
30 780 doi:10.1038/35051594
31 781 25 Bulters D, Gaastra B, Zolnourian A, *et al.* Haemoglobin scavenging
32 782 in intracranial bleeding: biology and clinical implications. *Nat Rev*
33 783 *Neurol* 2018;**14**:416–32. doi:10.1038/s41582-018-0020-0
34 784 26 Zhao X, Sun G, Ting SM, *et al.* Cleaning up after ICH: The role of
35 785 Nrf2 in modulating microglia function and hematoma clearance. *J*
36 786 *Neurochem* 2015;**133**:144–52. doi:10.1111/jnc.12974
37 787 27 Morris CM, Candy JM, Edwardson JA, *et al.* Evidence for the
38 788 localization of haemopexin immunoreactivity in neurones in the
39 789 human brain. *Neurosci Lett* 1993;**149**:141–4. doi:10.1016/0304-
40 790 3940(93)90756-B
41 791 28 Chen M, Regan RF. Time course of increased heme oxygenase
42 792 activity and expression after experimental intracerebral hemorrhage:
43 793 Correlation with oxidative injury. *J Neurochem* 2007;**103**:2015–21.
44 794 doi:10.1111/j.1471-4159.2007.04885.x
45 795 29 Harada N, Kanayama M, Maruyama A, *et al.* Nrf2 regulates
46 796 ferroportin 1-mediated iron efflux and counteracts

- 1
2
3 797 lipopolysaccharide-induced ferroportin 1 mRNA suppression in
4 798 macrophages. *Arch Biochem Biophys* 2011;**508**:101–9.
5 799 doi:10.1016/j.abb.2011.02.001
6
7 800 30 Sandberg M, Patil J, D'Angelo B, *et al.* NRF2-regulation in brain
8 801 health and disease: Implication of cerebral inflammation.
9 802 *Neuropharmacology* 2014;**79**:298–306.
10 803 doi:10.1016/j.neuropharm.2013.11.004
11
12 804 31 Pan H, Wang H, Zhu L, *et al.* Depletion of Nrf2 enhances
13 805 inflammation induced by oxyhemoglobin in cultured mice
14 806 astrocytes. *Neurochem Res* 2011;**36**:2434–41. doi:10.1007/s11064-
15 807 011-0571-6
16
17 808 32 Chen G, Fang Q, Zhang J, *et al.* Role of the Nrf2-ARE pathway in
18 809 early brain injury after experimental subarachnoid hemorrhage. *J*
19 810 *Neurosci Res* 2011;**89**:515–23. doi:10.1002/jnr.22577
20
21 811 33 Li T, Wang H, Ding Y, *et al.* Genetic elimination of Nrf2
22 812 aggravates secondary complications except for vasospasm after
23 813 experimental subarachnoid hemorrhage in mice. *Brain Res*
24 814 2014;**1558**:90–9. doi:10.1016/j.brainres.2014.02.036
25
26 815 34 Zhao XD, Zhou YT, Lu XJ. Sulforaphane enhances the activity of
27 816 the Nrf2-ARE pathway and attenuates inflammation in OxyHb-
28 817 induced rat vascular smooth muscle cells. *Inflamm Res*
29 818 2013;**62**:857–63. doi:10.1007/s00011-013-0641-0
30
31 819 35 Zhao X, Wen L, Dong M, *et al.* Sulforaphane activates the cerebral
32 820 vascular Nrf2–ARE pathway and suppresses inflammation to
33 821 attenuate cerebral vasospasm in rat with subarachnoid hemorrhage.
34 822 *Brain Res* 2016;**1653**:1–7. doi:10.1016/j.brainres.2016.09.035
35
36 823 36 Farrell B, Godwin J, Richards S, *et al.* The United Kingdom
37 824 transient ischaemic attack (UK-TIA) aspirin trial: Final results. *J*
38 825 *Neurol Neurosurg Psychiatry* Published Online First: 1951.
39 826 doi:10.1136/jnnp.54.12.1044
40
41 827 37 Rankin J. Cerebral Vascular Accidents in Patients over the Age of
42 828 60: III. Diagnosis and Treatment. *Scott Med J* Published Online
43 829 First: 1957. doi:10.1177/003693305700200604
44
45 830 38 Quinn TJ, Dawson J, Walters MR, *et al.* Reliability of the modified
46 831 rankin scale: A systematic review. *Stroke* Published Online First:
47 832 2009. doi:10.1161/STROKEAHA.109.557256
48
49 833 39 Jennett B, Bond M. ASSESSMENT OF OUTCOME AFTER
50 834 SEVERE BRAIN DAMAGE. A Practical Scale. *Lancet* Published
51 835 Online First: 1975. doi:10.1016/S0140-6736(75)92830-5
52
53 836 40 Jennett B, Snoek J, Bond MR, *et al.* Disability after severe head
54 837 injury: Observations on the use of the Glasgow Outcome Scale. *J*
55 838 *Neurol Neurosurg Psychiatry* Published Online First: 1981.
56 839 doi:10.1136/jnnp.44.4.285

- 1
2
3 840 41 Pace A, Mitchell S, Casselden E, *et al.* A subarachnoid
4 841 haemorrhage-specific outcome tool. *Brain* 2018;**141**:1111–21.
5 842 doi:10.1093/brain/awy003
6
7 843 42 Powell JH, Beckers K, Greenwood RJ. Measuring progress and
8 844 outcome in community rehabilitation after brain injury with a new
9 845 assessment instrument - The BICRO-39 scales. *Arch Phys Med*
10 846 *Rehabil* Published Online First: 1998. doi:10.1016/S0003-
11 847 9993(98)90265-9
12
13 848 43 van Heugten C, Rasquin S, Winkens I, *et al.* Checklist for cognitive
14 849 and emotional consequences following stroke (CLCE-24):
15 850 development, usability and quality of the self-report version. *Clin*
16 851 *Neurol Neurosurg* 2007;**109**:257–62.
17 852 doi:10.1016/j.clineuro.2006.10.002
18
19 853 44 Al-Tamimi YZ, Bhargava D, Feltbower RG, *et al.* Lumbar drainage
20 854 of cerebrospinal fluid after aneurysmal subarachnoid hemorrhage: a
21 855 prospective, randomized, controlled trial (LUMAS). *Stroke*
22 856 2012;**43**:677–82. doi:10.1161/STROKEAHA.111.625731
23
24 857 45 Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to
25 858 subarachnoid hemorrhage visualized by computerized tomographic
26 859 scanning. *Neurosurgery* 1980;**6**:1–
27 860 9.<http://www.ncbi.nlm.nih.gov/pubmed/7354892>
28
29 861 46 Naidech AM, Janjua N, Kreiter KT, *et al.* Predictors and impact of
30 862 aneurysm rebleeding after subarachnoid hemorrhage. *Arch Neurol*
31 863 2005;**62**:410–6. doi:10.1001/archneur.62.3.410
32
33 864 47 Zhao J, Kobori N, Aronowski J, *et al.* Sulforaphane reduces infarct
34 865 volume following focal cerebral ischemia in rodents. *Neurosci Lett*
35 866 2006;**393**:108–12. doi:10.1016/j.neulet.2005.09.065
36
37 867 48 Zhao X, Sun G, Zhang J, *et al.* Transcription factor Nrf2 protects
38 868 the brain from damage produced by intracerebral hemorrhage.
39 869 *Stroke* 2007;**38**:3280–6. doi:10.1161/STROKEAHA.107.486506
40
41 870 49 Alfieri A, Srivastava S, Siow RCM, *et al.* Sulforaphane
42 871 preconditioning of the Nrf2/HO-1 defense pathway protects the
43 872 cerebral vasculature against blood-brain barrier disruption and
44 873 neurological deficits in stroke. *Free Radic Biol Med* 2013;**65**:1012–
45 874 22. doi:10.1016/j.freeradbiomed.2013.08.190
46
47 875 50 Sharma V, McNeill JH. To scale or not to scale: The principles of
48 876 dose extrapolation. *Br. J. Pharmacol.* 2009. doi:10.1111/j.1476-
49 877 5381.2009.00267.x
50
51 878 51 Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal
52 879 to human studies revisited. *FASEB J* Published Online First: 2007.
53 880 doi:10.1096/fj.07-9574LSF
54
55 881 52 Clarke JD, Hsu A, Williams DE, *et al.* Metabolism and tissue
56 882 distribution of sulforaphane in Nrf2 knockout and wild-type mice.

- 1
2
3 883 *Pharm Res* 2011;**28**:3171–9. doi:10.1007/s11095-011-0500-z
- 4 884 53 Jazwa A, Rojo AI, Innamorato NG, *et al.* Pharmacological
5 885 Targeting of the Transcription Factor Nrf2 at the Basal Ganglia
6 886 Provides Disease Modifying Therapy for Experimental
7 887 Parkinsonism. *Antioxid Redox Signal* 2011;**14**:2347–60.
8 888 doi:10.1089/ars.2010.3731
- 9 889 54 Kumar G, Shahripour RB, Harrigan MR. Vasospasm on transcranial
10 890 Doppler is predictive of delayed cerebral ischemia in aneurysmal
11 891 subarachnoid hemorrhage: a systematic review and meta-analysis.
12 892 2016;**124**:1257–64. doi:10.3171/2015.4.JNS15428.
- 13 893 55 Tam AKH, Ilodigwe D, Li Z, *et al.* Global cerebral atrophy after
14 894 subarachnoid hemorrhage: a possible marker of acute brain injury
15 895 and assessment of its impact on outcome. *Acta Neurochir Suppl*
16 896 Published Online First: 2013. doi:10.1007/978-3-7091-1192-5_5
- 17 897 56 Nicci Campbell, Carl Verschuur, Sophie Mitchell, Orlaith
18 898 McCaffrey, Lewis Deane, Hannah Taylor, Rory Smith, Lesley
19 899 Foulkes, James Glazier, Angela Darekar, Mark E Haacke, Diederik
20 900 Bulters and IG. Hearing impairment after subarachnoid
21 901 haemorrhage. *Ann Clin Transl Neurol* 2018;**In press**.
22 902 doi:10.1002/acn3.714
- 23 903 57 Vergouwen MDI, Vermeulen M, van Gijn J, *et al.* Definition of
24 904 delayed cerebral ischemia after aneurysmal subarachnoid
25 905 hemorrhage as an outcome event in clinical trials and observational
26 906 studies: proposal of a multidisciplinary research group. *Stroke*
27 907 2010;**41**:2391–5. doi:10.1161/STROKEAHA.110.589275
- 28 908 58 Alaraj A, Hussein AE, Esfahani DR, *et al.* Reducing length of stay
29 909 in aneurysmal subarachnoid hemorrhage: A three year institutional
30 910 experience. *J Clin Neurosci* 2017;**42**:66–70.
31 911 doi:10.1016/j.jocn.2017.03.049
- 32 912 59 Teasdale GM, Drake CG, Hunt W, *et al.* A universal subarachnoid
33 913 haemorrhage scale: report of a committee of the world federation of
34 914 Neurosurgical societies. *J. Neurol. Neurosurg. Psychiatry*. 1988.
35 915 doi:10.1136/jnnp.51.11.1457
- 36 916 60 Jaja BNR, Saposnik G, Lingsma HF, *et al.* Development and
37 917 validation of outcome prediction models for aneurysmal
38 918 subarachnoid haemorrhage: The SAHIT multinational cohort study.
39 919 *BMJ* Published Online First: 2018. doi:10.1136/bmj.j5745
- 40 920
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Page No.	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2	Trial identifier and registry name. If not yet registered, name of intended registry
	2	All items from the World Health Organization Trial Registration Data Set
Protocol version	1	Date and version identifier
Funding	15	Sources and types of financial, material, and other support
Roles and responsibilities	1	Names, affiliations, and roles of protocol contributors
	1,15	Name and contact information for the trial sponsor
	15	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	13, 14, 15	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	3,4	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	4	Explanation for choice of comparators
Objectives	4,5	Specific objectives or hypotheses
Trial design	4,5	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	6	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	6,7	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	7,8	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	8	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	8	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	8	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	8,9,10	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	10,11	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	11	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	11	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	12	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
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1			
2	Allocation	12	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5			assigned
6			
7	Implementation	12	Who will generate the allocation sequence, who will enrol participants,
8			and who will assign participants to interventions
9			
10	Blinding	12	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12			how
13			
14			
15		12	If blinded, circumstances under which unblinding is permissible, and
16			procedure for revealing a participant's allocated intervention during
17			the trial
18			

Methods: Data collection, management, and analysis

21	Data collection	12	Plans for assessment and collection of outcome, baseline, and other
22	methods		trial data, including any related processes to promote data quality (eg,
23			duplicate measurements, training of assessors) and a description of
24			study instruments (eg, questionnaires, laboratory tests) along with
25			their reliability and validity, if known. Reference to where data
26			collection forms can be found, if not in the protocol
27			
28			
29			
30		N/A	Plans to promote participant retention and complete follow-up,
31			including list of any outcome data to be collected for participants who
32			discontinue or deviate from intervention protocols
33			
34	Data	13	Plans for data entry, coding, security, and storage, including any
35	management		related processes to promote data quality (eg, double data entry;
36			range checks for data values). Reference to where details of data
37			management procedures can be found, if not in the protocol
38			
39			
40	Statistical	12,	Statistical methods for analysing primary and secondary outcomes.
41	methods	13	Reference to where other details of the statistical analysis plan can be
42			found, if not in the protocol
43			
44		12	Methods for any additional analyses (eg, subgroup and adjusted
45			analyses)
46			
47		13	Definition of analysis population relating to protocol non-adherence
48			(eg, as randomised analysis), and any statistical methods to handle
49			missing data (eg, multiple imputation)
50			
51			

Methods: Monitoring

54	Data monitoring	13	Composition of data monitoring committee (DMC); summary of its role
55			and reporting structure; statement of whether it is independent from
56			the sponsor and competing interests; and reference to where further
57			details about its charter can be found, if not in the protocol.
58			Alternatively, an explanation of why a DMC is not needed
59			
60			

1			
2		13	Description of any interim analyses and stopping guidelines, including
3			who will have access to these interim results and make the final
4			decision to terminate the trial
5			
6	Harms	13,	Plans for collecting, assessing, reporting, and managing solicited and
7		14	spontaneously reported adverse events and other unintended effects
8			of trial interventions or trial conduct
9			
10	Auditing	13,	Frequency and procedures for auditing trial conduct, if any, and
11		14	whether the process will be independent from investigators and the
12			sponsor
13			
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Ethics and dissemination

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16			
17	Research ethics	2	Plans for seeking research ethics committee/institutional review board
18	approval		(REC/IRB) approval
19			
20	Protocol	2	Plans for communicating important protocol modifications (eg,
21	amendments		changes to eligibility criteria, outcomes, analyses) to relevant parties
22			(eg, investigators, REC/IRBs, trial participants, trial registries, journals,
23			regulators)
24			
25			
26	Consent or assent	14	Who will obtain informed consent or assent from potential trial
27			participants or authorised surrogates, and how (see Item 32)
28			
29		14	Additional consent provisions for collection and use of participant data
30			and biological specimens in ancillary studies, if applicable
31			
32	Confidentiality	12	How personal information about potential and enrolled participants will
33			be collected, shared, and maintained in order to protect confidentiality
34			before, during, and after the trial
35			
36			
37	Declaration of	15	Financial and other competing interests for principal investigators for
38	interests		the overall trial and each study site
39			
40	Access to data	15	Statement of who will have access to the final trial dataset, and
41			disclosure of contractual agreements that limit such access for
42			investigators
43			
44			
45	Ancillary and	14	Provisions, if any, for ancillary and post-trial care, and for
46	post-trial care		compensation to those who suffer harm from trial participation
47			
48	Dissemination	2	Plans for investigators and sponsor to communicate trial results to
49	policy		participants, healthcare professionals, the public, and other relevant
50			groups (eg, via publication, reporting in results databases, or other
51			data sharing arrangements), including any publication restrictions
52			
53		15	Authorship eligibility guidelines and any intended use of professional
54			writers
55			
56		N/A	Plans, if any, for granting public access to the full protocol, participant-
57			level dataset, and statistical code
58			
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Appendices N/A

Informed consent materials	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.

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