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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-028514
Article Type:	Protocol
Date Submitted by the Author:	13-Dec-2018
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Keywords:	Randomised controlled trial, Subarachnoid haemorrhage, Nrf2, Sulforaphane, Delayed cerebral ischaemia

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

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].

Nrf2 also upregulates haptoglobin (Hp), an acute phase glycoprotein found in plasma [16], as well as cerebrospinal fluid (CSF) [17]. Hp is part of an important Hb scavenging pathway after SAH. It tightly binds to free Hb, and this Hb-Hp complex is taken up by CD163-positive macrophages [18]. This pathway is saturated after SAH [17] and upregulation of Hp represents a possible therapeutic avenue [19]. Nrf2 also regulates degradation of red blood cells, and metabolism of haem and iron through transcriptional upregulation of CD36 [20] [20], haemopexin [21], HO-1 [22] and ferritin [23].
Nrf2 is expressed in the central nervous system (CNS) and is upregulated in response to inflammation and cerebral insults [24]. Nrf2 knockout is associated with a more pronounced inflammatory response *in vitro* [25] and *in vivo* [26], and the increased inflammatory response is associated with more brain oedema, cell death and poorer neurological recovery [27]. SFN increases HO-1, NQO1, and GST-α1 levels and reduces IL-1β, IL-6, and TNF-α [28]. It leads to a reduction in vasospasm and improves neurological recovery [26] [29].

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

Objectives

The objective of the study is to assess the safety, pharmacokinetics and efficacy of SFX-01.

Methods

Trial design

This is a prospective, double-blind, parallel group, randomised controlled trial comparing SFX-01 (300 mg) taken orally as capsules or as a suspension via a nasogastric tube (NG) twice-daily for up to 28 days *versus* placebo in patients with SAH within 48 hours of enrolment.

The treatment window was selected based on clinical and biochemical criteria. After SAH red blood cells in the subarachnoid space lyse and gradually release Hb. This lysis takes time and initially any free haemoglobin is bound by Hp. Only with progressive Hb accumulation do levels of free Hb rise with the most marked increase occurring after three days [30]. Clinically delayed cerebral ischaemia (DCI) is only seen after three days. Therefore, initiation of treatment should ideally occur within the first 72 hours, to allow more time for Nrf2 pathway activation and expression of its transcriptome. However not all patients present and/or are diagnosed immediately. Since a local audit at the main study neurosurgical centre showed that most patients are admitted within 48 hours, initiation of treatment within 48 hours was established

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 ≥2.5mg/dL (221 μmol/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

SFX-01 (active 300 mg capsule) or placebo (cyclodextrin only capsule) will be taken orally or as a suspension via a nasogastric tube (NG) twice daily for up to 28 days.

Animal studies in ischaemic stroke, intracerebral haemorrhage and SAH have all used 5 mg/kg dose of SFN in rodents [26] [41] [42] [43]. Conversion of animal doses to humans using body surface area, as has been widely recommended [44] [45], yields a human dose of 50 mg SFN. This is equivalent to 300 mg of SFX-01 containing 46.15 mg of SFN. In the clinical studies conducted to date, SFX-01 has been shown to be well tolerated at doses of 600mg once daily and 300mg twice daily with no serious adverse effects.

De-escalation from the trial regimen

In the event of tolerability problems whilst the patient is in the neurosurgical centre, the Investigator will assess whether simple measures to ease the effects of the adverse event(s) may be implemented (i.e. antacid in the case of GI irritation or anti-emetic in the event of nausea).
The investigator will also assess whether or not the adverse event(s) could be related to the trial medication and severe enough to warrant a dose frequency reduction. In the first instance the investigator may consider missing one dose. If a dose frequency reduction is warranted, from that point onwards the second dose of the day will be omitted; a dose frequency increase back to twice daily will not be permitted.

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

Treatment compliance

Compliance with treatment will be recorded during the inpatient hospital stay by health care professionals and/or a member of the research team. On discharge to the usual residence, responsibility for this is transferred to the patient or their Personal Legal Representative, aided by detailed instructions. In the event of discharge to a rehabilitation unit or patient local hospital, written instructions will be provided on discharge and verbal communication with the clinical team will ensure compliance.

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

Concomitant treatment

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

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

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

MRI – iron and brain volume

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

Clinical outcome

- 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

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].

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] **Study procedures**	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)
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				
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

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

Consent will be obtained in one of three scenarios:

1- Patients with capacity
2- Patients without capacity, but with a relative or next of kin available immediately in person
3- Patients without capacity and no relative or next of kin immediately available in person. Professional legal representative will be approached.

Re-Consent

This must take place in two different scenarios:

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

Discussion

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

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

Footnotes

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

Funding: This study is sponsored by EvgenPharma.

Competing interests: None declared.

Ethics approval: South central Research Ethics Committee, Hampshire A, UK.
Word count: 4192

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

Date and version: 16th May 2018, Version 7

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Abstract

Introduction

Subarachnoid haemorrhage from a ruptured cerebral aneurysm carries high morbidity and mortality. Despite huge advances in techniques to secure the aneurysm, 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 is unstable. SFX-01 (Evgen Pharma) is a novel composition comprised of synthetic sulforaphane stabilised within an 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 aneurysmal subarachnoid haemorrhage in the previous 48 hours. 90 patients will be randomised to receive SFX-01 (300mg) or placebo twice-daily for up to 28 days.

Safety will be assessed using blood tests and adverse event reporting. Pharmacokinetics will be assessed based on paired blood and CSF sulforaphane levels on day seven. A subgroup will have 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 with mRS, GOSE, SAHOT, SF-36, BICRO-39 and CLCE-24. MRI at six months including quantitative susceptibility mapping and volumetric T1 will measure iron deposition and cortical volume.

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

Ethics and dissemination

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

Trial registration number: NCT02614742 ; Pre-results

Strengths and limitations of this study

- **A strength of this study is that it tests a new class of drug not previously used after human SAH.**
- **It is at low risk of bias due to placebo control and appropriate blinding.**
- **The study design includes multiple mechanistic outcomes to give deeper understanding of any clinical findings.**
- **The study includes multiple novel outcome measures and MRI sequences. While these may provide new insights, some are exploratory due to limited experience with them in SAH to date.**
- **It is a multicentre trial, and its results should be generalizable to patients with high volume SAH in neurosurgical units in the UK, although 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 and 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 of injury following SAH is multifactorial[8]. Early brain injury (EBI) refers to the processes occurring within the first 72 hours which include blood-brain barrier (BBB) dysfunction [9], cerebral oedema [10][11], neuronal cell death [12], altered ionic homeostasis, excitotoxicity, thrombin activation [13], vascular integrity degradation [14], oxidative stress [15], and inflammation [16]. However, despite the terminology, mechanisms such as oxidative stress and inflammation are not limited to this early period. They continue to worsen beyond the first three days, at the same time as CSF free haemoglobin (Hb) concentration rises markedly as it is released form the clot and mechanisms to dispose of Hb are saturated. It is also in this delayed phase when cerebral vasospasm occurs, affecting both micro- [17] and microvasculature [18].

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 [19] [20]. Some of these enzymes include

glutathione S-transferases (GSTs), NAD(P)H-quinone oxidoreductase 1 (NQO1) and haem oxygenase 1 (HO-1) [19]. 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 [21].

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

Nrf2 is expressed in the central nervous system (CNS) and is upregulated in response to inflammation and cerebral insults [30]. Nrf2 knockout is associated with a more pronounced inflammatory response *in vitro* [31] and *in vivo* [32], and the increased inflammatory response is associated with more brain oedema, cell death and poorer neurological recovery [33]. SFN increases HO-1, NQO1, and GST-α1 levels and reduces IL-1β, IL-6, and TNF-α [34]. It leads to a reduction in vasospasm and improves neurological recovery [32] [35].

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

Objectives

The objective of the study is to assess the safety, pharmacokinetics and efficacy of SFX-01.

Methods

Trial design

This is a prospective, double-blind, parallel group, randomised controlled trial comparing SFX-01 (300 mg) taken orally as capsules or as a suspension via a nasogastric tube (NG) twice-daily for up to 28 days *versus* placebo in patients with SAH within 48 hours of enrolment.

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

hospital. This would give optimal protection against early brain injury. Initiating treatment would still be justified up to 72 hours post-ictus, after which delayed cerebral ischaemia (DCI) occurs, such that a delay of more than 72 hours would be expected to undermine treatment efficacy. Within the initial 72 hours, earlier treatment would allow more time for Nrf2 pathway activation and expression of its transcriptome to protect against delayed events, as well as provide more opportunity for SFX-01 to act against early brain injury. On the other hand the earlier one stipulates treatment would start, the more patients one will exclude from the study. No animal studies have been conducted to investigate the timing of sulforaphane administration, and even if available, extrapolation of timing from animal models has its limitations due to the much quicker clot resorption in rodents. After considering all these factors, it was decided to start SFX-01 treatment at the earliest possible opportunity after SAH, yet still allow patients to be included if their presentation was delayed to some extent, to ensure generalizability to real clinical practice where delays in admission to tertiary centres are not uncommon. An audit of the lead study centre showed that most patients are admitted within 48 hours, leading to the adoption of SAH within 48 hours as the inclusion criterion, striking the best compromise between the practicality of recruitment and the need to start treatment early.

Detailed pharmacovigilance will inform the safety of SFX-01. Pharmacokinetics will be determined by measuring levels of SFN and its metabolites in CSF and blood at day seven in all patients; a more detailed profile will be obtained in a subgroup of up to 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) ([36] [37] [38] , Glasgow Outcome Score (GOSE) [39][40] and the SAH Outcome Tool (SAHOT) [41] as well as at day 90 and 180 using the mRS, GOSE, SAHOT, Brain Injury Community Rehabilitation Outcome Scales (BICRO-39) [42] and the Check List for Cognitive and Emotional consequences following stroke (CLCE-24) [43].

Patient and public involvement

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

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 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 ≥2.5mg/dL (221 µmol/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 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.

Inclusion of patients with unsecured aneurysms would risk a high incidence of rebleeding in the study. Rebleeding is associated with exceedingly bad outcomes [46], masking any effect from SFX-01. However, since not all aneurysms are secured within 48 hours, there will be 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 will be excluded.

Intervention

Trial medication

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

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

De-escalation from the trial regimen

In the event of tolerability problems whilst the patient is in the neurosurgical centre, the Investigator will assess whether simple measures to ease the effects of the adverse event(s) may be implemented (i.e. antacid in the case of GI irritation or anti-emetic in the event of nausea).
The investigator will also assess whether or not the adverse event(s) could be related to the trial medication and severe enough to warrant a dose frequency reduction. In the first instance the investigator may consider missing one dose. If a dose frequency reduction is warranted, from that point onwards the second dose of the day will be omitted; a dose frequency increase back to twice daily will not be permitted.

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

Treatment compliance

Compliance with treatment will be recorded during the inpatient hospital stay by health care professionals and/or a member of the research team. On discharge to the usual residence, responsibility for this will be transferred to the patient or their Personal Legal Representative, aided by detailed instructions. In the event of discharge to a rehabilitation unit or patient local hospital, written instructions will be provided on discharge and verbal communication with the clinical team will ensure compliance.

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

Concomitant treatment

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

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 will be one of the main objectives of this study. This will be 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. Adverse events will be coded following MEDDRA and followed up until resolution and graded for severity. Incidence of dose de-escalation or discontinuation will also be reported.

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 [52] [53] 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 and blood samples for six hours after dosing on days

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

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 [54].

Secondary end-points

Pharmacodynamic

CSF and blood – Haptoglobin and Malondialdehyde

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

MRI – iron and brain volume

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

Clinical outcome

- 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]

- 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

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 [41] 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 [41]. All these tools will be administered by research nurses or doctors trained in mRS and GOSE in person, or in the event this is not feasible, by phone.

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

Schedule of assessments

Time points[1] **Study procedures**	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)
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.

All treatment packs will be otherwise identical in appearance. Placebo capsules will be identical and contain α-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 from either the clinical trials pharmacy, or a suitably calibrated and monitored fridge outside the pharmacy. 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 decision to unblind will be made in discussion between the treating clinician and PI and where possible CI. 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.

Last observation carried forwards (LOCF) will be used to impute missing outcome at 6 months. 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 the safety aspect of the trial throughout. The DSMB will be independent of the study team and company and has its own charter. A steering committee (consisting of the Chief Investigator and the sponsor's Chief Medical Officer) will receive and review the reports from DSMB, and take action as appropriate.

The first 20 patients will only be dosed as an inpatient and may therefore have courses shorter than 28 days. The DSMB plan to hold an initial meeting after recruitment of 20 patients who will have completed seven days of trial medication. If there are no safety concerns, further patients will be allowed to be dosed with the trial drug after discharge to other hospitals or home. 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 deems 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.

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

Adverse events reporting

All 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.

Data Availability

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

Ethical considerations and Informed Consent

Consent procedures and emergency dosing

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

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

Consent will be obtained in one of three scenarios:

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

Re-Consent

This must take place in two different scenarios:

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

Discussion

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

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

Footnotes

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

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

Funding: This study is funded and sponsored by EvgenPharma.

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

Ethical approval: South Central Research Ethics Committee, Hampshire A, UK.
Word count: 4720

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

Date and version: 16th May 2018, Version 7

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Abstract

Introduction

Subarachnoid haemorrhage from a ruptured cerebral aneurysm carries high morbidity and mortality. Despite huge advances in techniques to secure the aneurysm, 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 is unstable. SFX-01 (Evgen Pharma) is a novel composition comprised of synthetic sulforaphane stabilised within an 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 aneurysmal subarachnoid haemorrhage in the previous 48 hours. 90 patients will be randomised to receive SFX-01 (300mg) or placebo twice-daily for up to 28 days.

Safety will be assessed using blood tests and adverse event reporting. Pharmacokinetics will be assessed based on paired blood and CSF sulforaphane levels on day seven. A subgroup will have 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 with mRS, GOSE, SAHOT, SF-36, BICRO-39 and CLCE-24. MRI at six months including quantitative susceptibility mapping and volumetric T1 will measure iron deposition and cortical volume.

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

Ethics and dissemination

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

Trial registration number: NCT02614742 ; Pre-results

Strengths and limitations of this study

- **A strength of this study is that it tests a new class of drug not previously used after human SAH.**
- **It is at low risk of bias due to placebo control and appropriate blinding.**
- **The study design includes multiple mechanistic outcomes to give deeper understanding of any clinical findings.**
- **The study includes multiple novel outcome measures and MRI sequences. While these may provide new insights, some are exploratory due to limited experience with them in SAH to date.**
- **It is a multicentre trial, and its results should be generalizable to patients with high volume SAH in neurosurgical units in the UK, although 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 and 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 of injury following SAH is multifactorial[8]. Early brain injury (EBI) refers to the processes occurring within the first 72 hours which include blood-brain barrier (BBB) dysfunction [9], cerebral oedema [10][11], neuronal cell death [12], altered ionic homeostasis, excitotoxicity, thrombin activation [13], vascular integrity degradation [14], oxidative stress [15], and inflammation [16]. However, despite the terminology, mechanisms such as oxidative stress and inflammation are not limited to this early period. They continue to worsen beyond the first three days, at the same time as CSF free haemoglobin (Hb) concentration rises markedly as it is released form the clot and mechanisms to dispose of Hb are saturated. It is also in this delayed phase when cerebral vasospasm occurs, affecting both micro- [17] and microvasculature [18].

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 [19] [20]. Some of these enzymes include

glutathione S-transferases (GSTs), NAD(P)H-quinone oxidoreductase 1 (NQO1) and haem oxygenase 1 (HO-1) [19]. 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 [21].

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

Nrf2 is expressed in the central nervous system (CNS) and is upregulated in response to inflammation and cerebral insults [30]. Nrf2 knockout is associated with a more pronounced inflammatory response *in vitro* [31] and *in vivo* [32], and the increased inflammatory response is associated with more brain oedema, cell death and poorer neurological recovery [33]. SFN increases HO-1, NQO1, and GST-α1 levels and reduces IL-1β, IL-6, and TNF-α [34]. It leads to a reduction in vasospasm and improves neurological recovery [32] [35].

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

Objectives

The objective of the study is to assess the safety, pharmacokinetics and efficacy of SFX-01.

Methods

Trial design

This is a prospective, double-blind, parallel group, randomised controlled trial comparing SFX-01 (300 mg) taken orally as capsules or as a suspension via a nasogastric tube (NG) twice-daily for up to 28 days *versus* placebo in patients with SAH within 48 hours of enrolment.

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

with different temporal profiles. Since SAH is an acute unpredictable event, the earliest one would be able to start treatment is soon after ictus, on admission into hospital. This would give optimal protection against early brain injury. Initiating treatment would still be justified up to 72 hours post-ictus, after which delayed cerebral ischaemia (DCI) occurs, such that a delay of more than 72 hours would be expected to undermine treatment efficacy. Within the initial 72 hours, earlier treatment would allow more time for Nrf2 pathway activation and expression of its transcriptome to protect against delayed events, as well as provide more opportunity for SFX-01 to act against early brain injury. On the other hand the earlier one stipulates treatment would start, the more patients one will exclude from the study. No animal studies have been conducted to investigate the timing of sulforaphane administration, and even if available, extrapolation of timing from animal models has its limitations due to the much quicker clot resorption in rodents. After considering all these factors, it was decided to start SFX-01 treatment at the earliest possible opportunity after SAH, yet still allow patients to be included if their presentation was delayed to some extent, to ensure generalizability to real clinical practice where delays in admission to tertiary centres are not uncommon. An audit of the lead study centre showed that most patients are admitted within 48 hours, leading to the adoption of SAH within 48 hours as the inclusion criterion, striking the best compromise between the practicality of recruitment and the need to start treatment early.

Detailed pharmacovigilance will inform the safety of SFX-01. Pharmacokinetics will be determined by measuring levels of SFN and its metabolites in CSF and blood at day seven in all patients; a more detailed profile will be obtained in a subgroup of up to 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) ([36] [37] [38] , Glasgow Outcome Score (GOSE) [39][40] and the SAH Outcome Tool (SAHOT) [41] as well as at day 90 and 180 using the mRS, GOSE, SAHOT, Brain Injury Community Rehabilitation Outcome Scales (BICRO-39) [42] and the Check List for Cognitive and Emotional consequences following stroke (CLCE-24) [43].

Patient and public involvement

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

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 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 ≥2.5mg/dL (221 µmol/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 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. In addition to using Fisher scale, all baseline CT scans will undergo volumetric analysis of blood load, which are more objective and reliable than any versions of the Fisher scale.

Inclusion of patients with unsecured aneurysms would risk a high incidence of rebleeding in the study. Rebleeding is associated with exceedingly bad outcomes [46], masking any effect from SFX-01. However, since not all aneurysms are secured within 48 hours, there will be 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 will be excluded.

Intervention

Trial medication

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

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

De-escalation from the trial regimen

In the event of tolerability problems whilst the patient is in the neurosurgical centre, the Investigator will assess whether simple measures to ease the effects of the adverse event(s) may be implemented (i.e. antacid in the case of GI irritation or anti-emetic in the event of nausea).
The investigator will also assess whether or not the adverse event(s) could be related to the trial medication and severe enough to warrant a dose frequency reduction. In the first instance the investigator may consider missing one dose. If a dose frequency reduction is warranted, from that point onwards the second dose of the day will be omitted; a dose frequency increase back to twice daily will not be permitted.

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

frequency de-escalation (dropping to once daily) will not be carried out after discharge from the neurosurgical centre; if tolerability problems occur after discharge, medication will be stopped; patients will continue in the study and complete the study visits in accordance with the schedule of assessments.

Treatment compliance

Compliance with treatment will be recorded during the inpatient hospital stay by health care professionals and/or a member of the research team. On discharge to the usual residence, responsibility for this will be transferred to the patient or their Personal Legal Representative, aided by detailed instructions. In the event of discharge to a rehabilitation unit or patient local hospital, written instructions will be provided on discharge and verbal communication with the clinical team will ensure compliance.

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

Concomitant treatment

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

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 will be one of the main objectives of this study. This will be 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. Adverse events will be coded following MEDDRA and followed up until resolution and graded for severity. Incidence of dose de-escalation or discontinuation will also be reported.

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 [52] [53] 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 and blood samples for six hours after dosing on days one and seven. Hourly CSF sampling will be performed by trained study personnel using a bespoke sterile closed cascade of syringes so that the EVD line is accessed directly only once to reduce the risk of infection. Sample collection and processing are detailed in specific study operating procedures.

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 [54].

Secondary end-points

Pharmacodynamic

CSF and blood – Haptoglobin and Malondialdehyde

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

MRI – iron and brain volume

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

Clinical outcome

- 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]
- 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

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 [41] 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 [41]. All these tools will be administered by research nurses or doctors trained in mRS and GOSE in person, or in the event this is not feasible, by phone.

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

A summary of all the key study activities with their specific time points are outlined in table 1.

Table 1. Schedule of assessments

Time points[1] **Study procedures**	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)
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.

All treatment packs will be otherwise identical in appearance. Placebo capsules will be identical and contain α-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 from either the clinical trials pharmacy, or a suitably calibrated and monitored fridge outside the pharmacy. 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 decision to unblind will be made in discussion between the treating clinician and PI and where possible CI. 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.

Last observation carried forwards (LOCF) will be used to impute missing outcome at 6 months. 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 the safety aspect of the trial throughout. The DSMB will be independent of the study team and company and has its own charter. A steering committee (consisting of the Chief Investigator and the sponsor's Chief Medical Officer) will receive and review the reports from DSMB, and take action as appropriate.

The first 20 patients will only be dosed as an inpatient and may therefore have courses shorter than 28 days. The DSMB plan to hold an initial meeting after recruitment of 20 patients who will have completed seven days of trial medication. If there are no safety concerns, further patients will be allowed to be dosed with the trial drug after discharge to other hospitals or home. 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 deems 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.

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

Adverse events reporting

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

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

will be monitored closely by the research team and any concerns will be highlighted and addressed accordingly.

Data Availability

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

Ethical considerations and Informed Consent

Consent procedures and emergency dosing

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

Consent will be obtained in one of three scenarios:

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

Re-Consent

This must take place in two different scenarios:

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

Discussion

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

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

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

Footnotes

Authors' contributions:

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

Acknowledgements:

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

Funding:

This study is funded and sponsored by EvgenPharma.

Competing interests:

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

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

Word count: 5220

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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Allocation concealment mechanism	12	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	12	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	12	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	12	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	12	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	N/A	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	13	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	12, 13	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	12	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	13	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	13	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	13	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	13, 14	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	13, 14	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

Research ethics approval	2	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	2	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	14	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	14	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	12	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	15	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	15	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	14	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	2	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	15	Authorship eligibility guidelines and any intended use of professional writers
	N/A	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

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.