PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A study protocol for SFX-01 After Subarachnoid haemorrhage (SAS): A multi-centre randomised double-blinded, placebo controlled trial
AUTHORS	Zolnourian, Ardalan; Franklin, Stephen; Galea, Ian; Bulters, Diederik

VERSION 1 - REVIEW

REVIEWER	R Loch Macdonald University of Toronto, Canada
REVIEW RETURNED	26-Dec-2018

GENERAL COMMENTS	Bmjopen 2018 028514 Review This is a well done clinical trial description about the use of sulforaphane in subarachnoid hemorrhage. Some questions and comments that I have are: General comments - One thing about a protocol review is I don't feel like I do or should or have control over the study design and protocol itself although I have a bunch of comments. Some comments not for review Duration of drug treatment and followup. Drug CMC details, phase 1 trial data not published, what does cyclodextrin do? If sulforaphane is unstable, how does it get released from the cyclodextrin in the gut and then absorbed, passed through the liver, into the circulation and then in to the brain? The instability must be in the gut then? What was the role of Evgen? 48 hours is a good acute time frame to start treatment but it is more challenging to get patients randomized that quickly. Hats off to you if you can get that done and not have to exclude too many patients or have too many protocol deviations (randomized, but dose started after 48 hours). It reads like you are given a grace period of 24 hours so that certainly helps. What was the rationale for 180 days? I found in other studies that centers didn't see patients back after 3 months. 6 month followup would result in many lost to followup. Questions – These are things I think could be clarified or that would enhance the protocol presentation if you agree. 1. The dose and dose regimen and sample size are not in the abstract. 2. Fisher 3 and 4 in 1980 paper were 4 – thin or no SAH and ICH or IVH. Is this really the scale you used??

	3. Do the active and placebo look the same. What if cyclodextrin is not a placebo? It has pharmacologic actions or at least does something. Who made the capsules, where, what company, what are the CMC controls and such on the manufacture of CMC, maybe it is reported elsewhere or at least you can say you have the documentation, what is the supply chain to get the active/placebo to each site and how is this controlled as in what system is used – a web-based system from a company? Does temperature of the shipment matter? Etc. ?research pharmacies? Do the pharmacies operate 24/7/365 or will you miss patients on weekends or at night? 4. For PK, is it acceptable to do an LP just to obtain CSF for PK? Hourly sampling of EVD CSF also is risky as it could increase the risk of infection. Is there a protocol for EVD management, how is the CSF handled after collection? Is there a separate SOP for that and for analysis of the samples? 5. For clinical outcomes, well, some of these are radiologic, but they all need some more description such as who is administering the mRS, is a standardized questionare being used, are the assessors trained in using the mRS, how do you get patients assessed at 180 days, can it be by phone? Same for the other clinical outcomes. For the radiology, who is assessing the CT or MRI, what are the definition of new infarct, is that adjudicated by anyone else, who determines if it is due to DCI or not, as a primary or as a contributing cause? Does every patient get post aneurysm repair imaging and then a followup scan so you can tell what the new hypodensities are? What is the protocol defined indication for hypertensive therapy and what is it defined as? 6. A more fundamental question is how do you know the dose selected is correct and did you consider a dose ranging study of some type first? 7. Safety as primary outcome – do you need to better define or quantify the GI side effects. 8. For emergency unblinding, who decides to unblind and does the DMC weight in on the question. 9. The DMC plan sounds good but I wasn't sure how they could review the data of 20 patients and then allow them to be treated up to 28 days. It takes days to get all the data in and checked and prepared before the DMC can even review it. Is there a chair of the DMC and are the members independent of the study and company. The whole DMC meeting for SUSARS also could be difficult, I assume there is a DMC charter that goes over all this. What is an increase in grading of severity of Aes? 10. What is the system used for AE reporting and for severity and seriousness. Are the sites being audited or monitored and is there a monitoring plan? Are there any waved AE? 11. Maybe just clarify – Evgen is paying for the study? What is their role otherwise in the study? 12. Pregnancy seems to be an issue since there patients are excluded and also contraception needs to be used after. Do you need to do a pregnancy test before randomizing the patient? What about patients who are later found to have been pregnant during or prior to the study? 13. Handling of drop outs and lost to followup is unclear. For clinical measures are you using last observation carried forwards or imputing an outcome? Well, these are a few things I was thinking about. I assume there is a full protocol somewhere, usually industry sponsored studies have several hundred page protocols.

REVIEWER	Richrd F. Keep University of Michigan, USA
REVIEW RETURNED	24-Jan-2019

GENERAL COMMENTS	This manuscript describes the study protocol for a planned clinical trial of SFX-01 for subarachnoid hemorrhage (SAH). SFX-01 is a complex of sulforaphane (SFN) and cyclodextrin that can be given orally to deliver SFN to patients. There is considerable preclinical evidence that SFN can protect against a variety of neurological conditions, including SAH. It induces the transcription factor nrf2, a potent regulator of anti-oxidant and anti-inflammatory defense mechanisms. There is evidence for oxidative stress and neuroinflammation being involved in SAH-induced brain injury. I have the following comments on the manuscript: 1) In the abstract, it is important to clearly state the primary end-points and then the secondary endpoints. 2) Similarly, in the abstract, it is important to give the number of patients that will be recruited. That will give the reader a better insight into the extent of the trial. 3) There needs to be some discussion of early vs. delayed brain injury after SAH in the introduction. The investigators have chosen to initiate SFX-01 treatment up to 48 hours after ictus and focus on delayed cerebral ischemia (DCI) as a primary-end point. They appear to be primarily focusing on delayed brain injury but there is evidence the SFN may impact early injury after SAH (Chen et al. Ref 26), as well as in other neurological conditions. 4) The investigators should discuss any preclinical evidence that SFN is protective in SAH when administration is started 48 hours after ictus? 5) Have any of the SFX-01 clinical studies been published. If so, they should be cited (e.g. on page 7 margin line 14) 6) Minor points. On page 8 line 34, the investigators should mention that they will be taking hourly blood samples as well as the CSF samples. Also, at this point, the investigators state that up to 12 patients will undergo such sampling. Page 5 line 10 states 12 patients.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:
Reviewer: 1
Reviewer Name: R Loch Macdonald
Institution and Country: University of Toronto, Canada
Please state any competing interests or state ‘None declared’: None delcared

Please leave your comments for the authors below
Bmjopen 2018 028514 Review
This is a well done clinical trial description about the use of sulforaphane in subarachnoid hemorrhage. Some questions and comments that I have are:

General comments - One thing about a protocol review is I don’t feel like I do or should or have control over the study design and protocol itself although I have a bunch of comments. Some comments not for review

We have given explanations for each of these items for the reviewer's interest but our interpretation was that they were not necessarily for inclusion in the manuscript and have therefore not done so, but can if requested.

Duration of drug treatment and followup.

The first 20 patients before the planned safety review were dosed for the duration of their inpatient stay and stopped on discharge or day 28 whichever was sooner.

After the safety review patients were dosed as inpatient and continued after discharge up to their first follow up visit. We wanted patients to still be taking IMP at the time of blood sampling at the first study visit to be able to observe the effect of the IMP. Therefore, the window for first follow up was selected to be day 22-30 so that all patients would have a minimum of 21 days of treatment in line with the time course of vasospasm and most SAH trials, and a maximum of 28 days (if recruited 48 hours after SAH and then had 28 days of treatment).

Follow up was selected to mirror clinical practice in the participating units where all aneurysmal SAH patients have clinical follow-up at three months and attend for radiological follow-up at 6 months. Therefore day 90 and 180 have been planned to coincide with these which will enable the research team to liaise with the clinical team where necessary in addition to reducing loss to follow-up.

phase 1 trial data not published,

We have discussed this with Evgen Pharma who undertook these trials. There is no specific reason for the lack of publication other than to date this has not met with the company's priorities. They have been asked by several other sources why it has not been published and are therefore planning to do this going forwards.

what does cyclodextrin do?

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. This process has been patented by Evgen Pharma.

If sulforaphane is unstable, how does it get released from the cyclodextrin in the gut and then absorbed, passed through the liver, into the circulation and then in to the brain? The instability must be in the gut then?

The instability refers to the formulation of the sulforaphane ex vivo. Any preparation of SFN has a relatively short half life depending on the exact preparation making distribution and storage in a clinical setting impractical. When SFX-01 is ingested the sulforaphane is released from the cyclodextrin in the gut and is then absorbed.

What was the role of Evgen?

Evgen is the study funder and sponsor.
The trial was originally conceived by the chief investigator when looking for options to upregulate haptoglobin expression as a way to improve outcome after SAH. This identified the nrf-2 pathway and sulforaphane as the most promising. This led to the design of a protocol for a randomised controlled trial with which we approached Evgen pharma. Evgen Pharma subsequently accepted the proposal

and funded and sponsored the trial. They have since made significant revisions to the protocol and employed a study management team.

48 hours is a good acute time frame to start treatment but it is more challenging to get patients randomized that quickly. Hats off to you if you can get that done and not have to exclude too many patients or have too many protocol deviations (randomized, but dose started after 48 hours). It reads like you are given a grace period of 24 hours so that certainly helps.

Yes this is a challenging target. However, prior to the study we had audited our admission times and knew that the vast majority of our SAH patients were admitted within less than 48 hours and therefore gave ourselves the ambition to capture this entire population that would both be representative of most of our population and early enough to maximise the effects of SFX-01.

In practice this has not proved a problem (even without a 24 hour grace period which the protocol does not allow). However, this is only possible with the ethical permission to give the first dose without consent in patients without capacity and a dedicated research team working closely with the clinical team.

What was the rationale for 180 days? I found in other studies that centres didn't see patients back after 3 months. 6 month follow-up would result in many lost to follow-up.

In our unit all patients have a clinical follow-up at 3 months as mentioned but the majority will also undergo follow up MRI at 6 months. Therefore, we designed the timing of the follow-up in a way to incorporate the research interventions at the same time as the clinical visits. In our experience this has worked very well in previous studies, with almost all patients attending their follow ups so far.

Questions – These are things I think could be clarified or that would enhance the protocol presentation if you agree.

Yes, we have tried to incorporate all these comments.

1. The dose and dose regimen and sample size are not in the abstract.

Lines 69-70

This has been added in the abstract." 90 patients will be randomised to receive SFX-01 (300mg) or placebo twice daily for up to 28 days."

2. Fisher 3 and 4 in 1980 paper were 4 – thin or no SAH and ICH or IVH. Is this really the scale you used??

This is a good point. Unfortunately, we have used the original Fisher scale which represents what has been historically used in our unit rather than reflecting more up to date modifications. We have therefore not amended the manuscript. However, all baseline CT scans are undergoing volumetric analysis of blood load, which are far more reliable than any versions of the Fisher scale.

3. Do the active and placebo look the same.

Active and placebo look exactly the same. This is also the case for the capsule contents. This is important as patients with NG tube will have the contents of the capsule dissolved in normal saline and there is no distinct smell or difference in the powder of either placebo or active medication.

There was already reference to this under randomisation and blinding: “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.”

Lines 466-467

We have added “SFX-01 and placebo capsules as well as their contents will be identical in appearance” to the section trail medication.

What if cyclodextrin is not a placebo? It has pharmacologic actions or at least does something.

This is an interesting consideration. It is in our view more theoretical than practical. There is no literature to support cyclodextrin as a protective agent in SAH and it is widely used in the pharmaceutical industry as a delivery system and we have therefore not amended the text at this point.

Who made the capsules, where, what company,

Lines 464-465

We have added the response in the section trial medication “Pharmagra manufacture is the active pharmaceutical ingredient (API) in North Carolina, US. The API is then encapsulated at Quotient in Reading, UK.”

what are the CMC controls and such on the manufacture of CMC, maybe it is reported elsewhere or at least you can say you have the documentation, what is the supply chain to get the active/placebo to each site and how is this controlled as in what system is used – a web-based system from a company?

Lines 465-466

The API manufacture has been audited by the FDA and also by Evgen. They are subject to the same controls that any GMP manufacturer would be. This has been added to the section on trial medication “The API manufacture has been audited by the FDA and Evgen pharma.”

Does temperature of the shipment matter? Etc.?

Although SFX-01 is relatively stable it cannot be stored at room temperature for protracted periods of time. In the trial therefore it is being kept at 2-8 degrees Celsius, at all times. This is both prior to shipment of IMP to different sites as well as storage of IMP during their admission and even after discharge from the hospital. The IMP is initially stored in the research pharmacy and dispensed to an out of pharmacy research refrigerator in the intensive care unit. When a patient is enrolled onto the study the IMP is transferred to the ward refrigerator where the patient is admitted to. This is done using a cooling bag. The cooling bag is also used after the patient is discharged from the hospital to their place of residence or a different hospital.

Lines 467-468

We have added a statement under trial medication “They will be stored at 2-8 degrees Celsius.”

research pharmacies? Do the pharmacies operate 24/7/365 or will you miss patients on weekends or at night?

Our clinical trials pharmacy routinely operates between 8am-5pm Monday to Friday. While they do provide an on call out of hours service, dispensing via this still takes time and we were keen to minimise time to administration by any means possible.

Therefore, we have a dedicated out of pharmacy research fridge in the neurointensive care unit where most patients will be admitted to. This is monitored daily, calibrated annually and audited regularly by the clinical trials pharmacy team as well as the research team. When a patient is recruited, IMP is therefore allocated to the patient directly from the out of pharmacy fridge and means that out of hours recruitment is possible without an out of hours clinical trials pharmacy or any delay.

Lines 694-697

We have added the sentence “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.” to the section on randomisation and blinding.

4. For PK, is it acceptable to do an LP just to obtain CSF for PK?
Prior to undertaking the study, we assessed our historic data between 2012-2014 and identified all the patients who had CSF drainage for clinical purposes. We found that 25% had EVD and 30% had lumbar puncture. The rates are likely to be higher in a selected study cohort with high blood load. It was therefore anticipated that a large majority of patients would be having CSF diversion during their clinical course anyway.
The decision to include a lumbar puncture for study purposes was further justified by the LUMAS RCT (Lumbar drainage of cerebrospinal fluid after aneurysmal subarachnoid haemorrhage) which showed lumbar CSF drainage after SAH is associated with reduced DCI and symptomatic benefit with improved clinical outcomes at 3 months, although this effect was lost in long-term after 6 months. This would suggest that Fisher 3 and 4 patients are not harmed by CSF diversion and if anything, they are likely to gain short term benefit.
In practice, in this patient group, we measure the opening pressure and after removal of research samples, if needed we remove further CSF to achieve a closing pressure within the normal range. In our experience many patients have symptomatic benefit from this even with no overt hydrocephalus on CT or when LP would not have otherwise been contemplated.
The issue was discussed at the ethical review meeting who felt it was appropriately described in the patient information sheets and patients sign specifically to have an LP.
We also discussed this at a SAH focus group involving SAH survivors and their relatives. A series of questions were put forward including whether they would participate in a trial which included lumbar puncture and the overwhelming majority indicated they would.

Line 309-313

We refer to this in the Patient and public involvement section “The overall design of the study including intended 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 comprising of those with previous history of SAH as well as their relatives.” But have added to this “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.”

Hourly sampling of EVD CSF also is risky as it could increase the risk of infection. Is there a protocol for EVD management, how is the CSF handled after collection?

This was another concern that was discussed extensively prior to the study. Although there is evidence that CSF sampling increases EVD infection rates in clinical practice, there is also good evidence there is no increase in risk of sampling in research practice, presumably due to strict protocols adhering to aseptic techniques.

We were not aware of reports of hourly CSF collection prior to the study. We therefore designed a specific SOP for this. Key to this was construction of a sterile closed sampling system compromising of eight syringes connected via two octopus connectors. This allowed the main EVD sampling port to be accessed once, yet seven samples to be obtained (one port was for aspiration of dead space) Furthermore, the hourly CSF collection was restricted to four investigators all of whom received study specific training.

Lines 556-559

We have added a sentence under pharmacokinetic “Hourly CSF sampling will be performed by trained study personnel using a bespoke sterile closed cascade of syringes so that EVD line is accessed directly only once to reduce the risk of infection.”

Is there a separate SOP for that and for analysis of the samples?

Lines 558-559

Yes, there is a a study SOP for sample collection and sample processing. We have added “Sample collection and processing are detailed in specific study operating procedures.”

5. For clinical outcomes, well, some of these are radiologic, but they all need some more description such as who is administering the mRS, is a standardized questionare being used, are the assessors trained in using the mRS,

The scales including mRS and GOSE are administered by research nurses/fellows. They have all been trained in using mRS through completing the mRS assessment through University of Glasgow (similar to that employed in MISTIE-III) and specific face to face GOSE training has been given by experts in the scale (similar to that employed in the NOSTRA-III trial).

All other standardised functional questionnaires are filled in by the patients or their carers. If this is not possible a member of research team will go through them with the patient/carers at the time of follow-up.

Lines 621-622

We have added a statement” All these tools are administered by research nurses or doctors trained in mRS and GOSE.”

how do you get patients assessed at 180 days, can it be by phone? Same for the other clinical outcomes.

At 180 days most patients normally attend a clinical MRI to which additional research sequences have been added. Only a minority would not normally attend for MRI and have the MRI for research purposes only. Clinical assessment is therefore done face to face before the MRI appointment and where this is not feasible a telephone follow up is performed.

Lines 622-623

We have added" in person, or in the event this is not feasible by phone."

For the radiology, who is assessing the CT or MRI, what are the definition of new infarct, is that adjudicated by anyone else, who determines if it is due to DCI or not, as a primary or as a contributing cause? Does every patient get post aneurysm repair imaging and then a followup scan so you can tell what the new hypodensities are?

A blinded consultant neuroradiologist will review the imaging of all patients. They will have access to the baseline CT (or any other CTs or perfusion CT that may have been done during the inpatient stay) and compare them against the follow-up MRI.

Lines 626-627

We have added "as adjudicated by a blinded consultant neuroradiologist (with baseline CT and follow up MRI)".

What is the protocol defined indication for hypertensive therapy and what is it defined as?

Hypertensive therapy is taken as institution of inotropes to increase blood pressure in intensive care.

6. A more fundamental question is how do you know the dose selected is correct and did you consider a dose ranging study of some type first?

A dose ranging study was considered. However, in animal studies 5mg/Kg of SFN has shown beneficial effects. Based on surface area, the human equivalent dose is 50mg. This coincides with the highest dose at which SFX-01 displayed no side effects in the phase 1 trials (300mg of SFX-01 contains 46.15mg of SFN). It was therefore hard to justify either a higher or a lower dose and hence a dose ranging study was not undertaken. This is already described under the section trial medication.

Lines 470-476

"Animal studies in ischaemic stroke, intracerebral haemorrhage and SAH have all used 5 mg/kg dose of SFN in rodents [31] [46] [47] [48]. Conversion of animal doses to humans using body surface area, as has been widely recommended [49] [50], 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."

Line 476

We have added to this "Therefore no further dose ranging studies were performed."

7. Safety as primary outcome – do you need to better define or quantify the GI side effects.

Safety will be assessed based on adverse event reporting as would be normal practice in a CTIMP study. The adverse events will be coded using standard MEDDRA coding with grouping by system organ class. This will include incidence of individual GI AEs like nausea, vomiting, abdominal pain etc. Time to onset of the event after starting drug will help inform causality and all AE's will be graded in severity (mild, moderate or severe) and followed up to resolution with duration of symptoms acting as another surrogate for severity.

Additionally, there is a de-escalation mechanism available in patients who are overly symptomatic and that will be used as a marker of severity of the side effects.

Line 538-540

We have added under safety “Adverse events will be coded following MEDDRA and followed until resolution and graded for severity. Incidence of dose de-escalation or discontinuation will also be reported “.

8. For emergency unblinding, who decides to unblind and does the DMC weight in on the question.

The treating clinician or study investigator would discuss with the PI and where possible the CI if unblinding would alter the clinical management of the patient and will make a decision based on this. The DMC would in an emergency setting for an individual patient would not participate in this decision.

Lines 706-708

We have added to the unblinding section” The decision to unblind will be made in discussion between the treating clinician and PI and where possible CI. The decision to unblind will be made in discussion between the treating clinician and PI and where possible CI.”

9. The DMC plan sounds good but I wasn’t sure how they could review the data of 20 patients and then allow them to be treated up to 28 days. It takes days to get all the data in and checked and prepared before the DMC can even review it.

We apologise that the text was not clear; the reviewer is correct and this is not possible. The plan is that the first 20 patients will be dosed only as an inpatient and if discharged before 28 days their course will be shorter. This is why the study description is of “up to 28 days” and not “28 days”. After these 20 patients the DMC occurs and then all subsequent patients will be dosed continuing after discharge if required.

We have now revised the section on monitoring to state “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.” which hopefully makes it clearer.

Is there a chair of the DMC and are the members independent of the study and company. The whole DMC meeting for SUSARS also could be difficult, I assume there is a DMC charter that goes over all this.

Lines 745-746

We have added” The DSMB is independent of the study team and company and has its own charter.”

What is an increase in grading of severity of Aes?

The severity of AEs are graded as follows: mild, moderate and severe. The severity and resolution of all AEs are reviewed at each follow up and if the severity is upgraded i.e from moderate to severe then this will initiate a DMC meeting.

As mentioned in the previous section, we have added information about grading and duration to the section on safety.

10. What is the system used for AE reporting and for severity and seriousness. Are the sites being audited or monitored and is there a monitoring plan?

Once again due to word restriction we opted to remove parts of this section which would have otherwise included all the items where further clarification has been requested. 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 (The Clinical Trials Company). All data are also entered onto an electronic case report form where further external monitoring will be done.

Lines 764-767

We have added to the section on monitoring" 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. "

Are there any waved AE?

There are no waived AEs and all AEs are logged.
We have changed "adverse events" to "all adverse events" under the heading Adverse events reporting.

11. Maybe just clarify – Evgen is paying for the study? What is their role otherwise in the study?

This role has been explained in response to the editor's comment above.

12. Pregnancy seems to be an issue since there patients are excluded and also contraception needs to be used after. Do you need to do a pregnancy test before randomizing the patient?

While there are some studies suggesting there is no harmful effect of the drug on sperm or ova, full reproductive studies have not been undertaken yet and and therefore although there are no known risks to the foetus, pregnant patients are excluded. Therefore, a pregnancy test (either blood or urine) is required. This is in the full schedule of events but had been removed from the abbreviated one in the manuscript. We have added it to this again. For further clarity we have added this in the schedule of events.

What about patients who are later found to have been pregnant during or prior to the study?

The expectation is that the incidence of missed pregnancy with modern testing will be exceedingly low, and we have audited the incidence of pregnancy in our past patients in the first 28 days after SAH (when patients would be taking IMP) and have only one case in the past ten years. We therefore hope if contraception is recommended this will not occur.

In the event it does occur, patients will be referred for close obstetric monitoring. All obstetric visits will be monitored as described under adverse events reporting.

13. Handling of drop outs and lost to followup is unclear. For clinical measures are you using last observation carried forwards or imputing an outcome?

We have detailed this in our local statistical analysis plan (SAP). For mRS and GOSE we have stated in the SAP we will potentially use last observation carried forwards (LOCF) to impute missing outcome at 6 months. The SAP states this will happen if there is more than 15% missing data in either arm, and also states if the results are marginal, the LOCF may be performed with less missing data.

Lines 737-738

We have added this to the section statistical analysis “Last observation carried forwards (LOCF) will be used to impute missing outcome at 6 months.”

Well, these are a few things I was thinking about. I assume there is a full protocol somewhere, usually industry sponsored studies have several hundred page protocols.

Yes there is a full protocol, Study operations manual, Study sampling manual, MRI manual, DSMB charter, and statistical analysis plan available amongst others.

Reviewer: 2
Reviewer Name: Richrd F. Keep
Institution and Country: University of Michigan, USA
Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below
This manuscript describes the study protocol for a planned clinical trial of SFX-01 for subarachnoid hemorrhage (SAH). SFX-01 is a complex of sulforaphane (SFN) and cyclodextrin that can be given orally to deliver SFN to patients. There is considerable preclinical evidence that SFN can protect against a variety of neurological conditions, including SAH. It induces the transcription factor nrf2, a potent regulator of anti-oxidant and anti-inflammatory defence mechanisms. There is evidence for oxidative stress and neuroinflammation being involved in SAH-induced brain injury.
I have the following comments on the manuscript:

1) In the abstract, it is important to clearly state the primary end-points and then the secondary endpoints.

The following has been amended to include the statement

Lines 84-85

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

2) Similarly, in the abstract, it is important to give the number of patients that will be recruited. That will give the reader a better insight into the extent of the trial.

Line 69
This has been added:“90 patients will be randomised to receive SFX-01”

3) There needs to be some discussion of early vs. delayed brain injury after SAH in the introduction.

We have rewritten the third paragraph:

Lines 149-159

“The mechanism of injury following SAH is multifactorial. Early brain injury (EBI) refers to the processes occurring within the first 72 hours which include blood-brain barrier (BBB) dysfunction [8], cerebral oedema [9][10], neuronal cell death [11], altered ionic homeostasis, excitotoxicity, thrombin activation [12], vascular integrity degradation [13], oxidative stress [14], and inflammation [15]. 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- [16] and microvasculature [17]”.

The investigators have chosen to initiate SFX-01 treatment up to 48 hours after ictus and focus on delayed cerebral ischemia (DCI) as a primary-end point. They appear to be primarily focusing on delayed brain injury but there is evidence the SFN may impact early injury after SAH (Chen et al. Ref 26), as well as in other neurological conditions.

SFX-01 has effects on multiple pathways after SAH - some in the early phase after SAH and some in the delayed and others in both phases. We are not specifically targeting either early phase or late phase. Practically our objective is to dose patients at the earliest possible time and so ameliorate both but accept that not all patients get to a neurosurgical centre immediately and therefore in some patients later administration is necessary in whom there will be more emphasis on delayed mechanisms. This is why we have obtained permission from the ethical committee to give an emergency dose without consent so as to ensure the earliest possible inclusion of patients in the study.

We have now made this clearer in paragraph 2 of trial design which reads

Lines 251-280

“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.”

4) The investigators should discuss any preclinical evidence that SFN is protective in SAH when administration is started 48 hours after ictus?

Lines 274-277

There are no studies of this which is acknowledged in paragraph 2 of trial design “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.”

5) Have any of the SFX-01 clinical studies been published. If so, they should be cited (e.g. on page 7 margin line 14)

These have not been published yet as discussed in the response to the other reviewer.

6) Minor points. On page 8 line 34, the investigators should mention that they will be taking hourly blood samples as well as the CSF samples. Also, at this point, the investigators state that up to 12 patients will undergo such sampling. Page 5 line 10 states 12 patients.

These have been addressed accordingly.

VERSION 2 – REVIEW

REVIEWER	R. Loch Macdonald University of Toronto, Canada
REVIEW RETURNED	24-May-2019

GENERAL COMMENTS	The revision is very well done. No further comments.

REVIEWER	Richard F. Keep Department of Neurosurgery, University of Michigan, USA
REVIEW RETURNED	27-May-2019

GENERAL COMMENTS	My critique has been addressed