PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A single-arm, open-label, dose-escalation Phase I study to evaluate the safety of an herbal medicine SH003 in patients with solid cancer: a study protocol
AUTHORS	Cheon, Chunhoo; Kang, Sohyeon; Ko, Youme; Kim, Mia; Jang, Bo-Hyoung; Shin, Yong-Cheol; Ko, Seong-Gyu

VERSION 1 – REVIEW

REVIEWER	Qingxi Yue Department of Oncology, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 201999, P.R. China
REVIEW RETURNED	12-Oct-2017

GENERAL COMMENTS	No comments

REVIEWER	ALESSANDRO COMANDONE ONCOLOGY DEPT, OSPEDALE HUMANITAS GRADENIGO, TORINO, ITALY
REVIEW RETURNED	31-Dec-2017

GENERAL COMMENTS	This is an uncommon phase I study on herbal compound in Clinical oncology SH 003 is a mixed herbal extract containing 3 different substances from traditional Korean medicine. This study is quite different from the abitual Phase I studies on citotoxic drugs or targeted agents we are used to read or to partecipate. I resume the limits of the protocol: 1) All the preclinical studies were done on tumoral cultured cells . Only Kim HY (Ref 20) performed a study on xenographt models. This limitation is far from the usual discovery and development process for a new drug in Oncology. 2) The starting dose in human studies has not been clearly determined. As a matter of fact the three levels of doses are very different: 1200 mg/day; 2400 mg/day; 4800 mg/day. 3) Furthermore: if the SH 003 tablet is 800 mg (see Interventions session)how can be administered 1200 mg / day (800 mg + 400 mg?) or only active substances (400 mg per tablets in 1.1.1 ratio) are considered to determine the total administered dose? 4) The study is presented as a dose excalation study following the 3+3 design. Does the model follow the Fibonacci's scheme or other models? The dose levels are determined with the well known dose excalation of 100%-67%-50%- 40%-33%? Or using different percentage steps?). All this aspects are not well defined in the "sample size" paragraph.

	5) Which are the expected Dose Limiting Toxicities? Which are the expected serious adverse effects? No data come from the preclinical studies session. 6) In common Phase I studies on citotoxic or biological drugs Pharmacokinetic's studies are fundamental. In this protocol the Pharmacokinetics investigations are not considered as correctly stated in the discussion part, but this is a clear limitation. In conclusion this protocol is interesting and as written by the Authors " is the first Phase I study on herbal medicine in Korea" and has to be considered by BMJ . On the other in order to promote the development of natural and herbal medicine the points listed above(1 to 6) should be considered to improve the reproducibility and the reliability of the study.

REVIEWER	Roberto Passera Dept. Nuclear Medicine, San Giovanni Battista University Hospital, University of Torino-I
REVIEW RETURNED	08-Jan-2018

GENERAL COMMENTS	- how have you selected the scaling rule (1200-2400-4800 mg SH003), which is quite different from the classical Fibonacci's one? - why the minumin age was equal to 19 yrs and not 18, as intl standard? Korean legal age? - every solid cancer? every previous chemo regimen? no limitations? in that case, why have you excluded oncohematological neoplasms? - no expected AEs are listed, which type of AEs according to CTCAE are you expecting? - "MTD will be defined as that dose level at which more than 1 out of 6 patients exhibit dose limiting toxicity during the 4 weeks..." IMHO, this is DLT definition, while MTD one is the last previous dose level, do you agree? - you should report descriptive stats for continuous variables as median(IQR) and for categorical ones as abs/rel frequencies

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:
Reviewer: 1
No comments

Reviewer: 2
This is an uncommon phase I study on herbal compound in Clinical oncology
SH 003 is a mixed herbal extract containing 3 different substances from traditional Korean medicine.
This study is quite different from the abitual Phase I studies on citotoxic drugs or targeted agents we are used to read or to partecipate.
I resume the limits of the protocol:

1) All the preclinical studies were done on tumoral cultured cells . Only Kim HY (Ref 20) performed a study on xenographt models. This limitation is far from the usual discovery and development process for a new drug in Oncology.
- Our response: Thank you for your thoughtful advice on the manuscript. We apologize that we have not fully described the preclinical studies. There were several xenograft studies on SH003. We have added relevant description to the manuscript. Although there are still some insufficiency in the

preclinical studies, we are working on further research as developing SH003, and the results also will be published in scientific journals.
We have added the following sentence to the introduction section: “In vivo xenograft studies have reported that SH003 inhibits tumour growth and metastasis, as well as VEGF-induced tumour angiogenesis without detectable toxicity, and SH003 in combination with doxorubicin has shown a synergistic effect in treating triple-negative breast cancer (TNBC).”

2) The starting dose in human studies has not been clearly determined. As a matter of fact the three levels of doses are very different: 1200 mg/day; 2400 mg/day; 4800 mg/day.
- Our response: We have added following sentences to the treatment protocol section: “It should be noted that no abnormal findings related to the investigational product were observed in either the single-dose toxicity study or the repeated-dose toxicity study. Therefore, the no observed adverse effect level (NOAEL) of the investigational product was determined to be 2500 mg/kg for rats. According to the FDA guideline, the maximum recommended starting dose for adults is 2400 mg per day based on a safety factor of 10. Based on the toxicity and efficacy study results, the starting dose was determined to be 1200 mg per day for this study.”

3) Furthermore: if the SH 003 tablet is 800 mg (see Interventions session)how can be administered 1200 mg / day (800 mg + 400 mg?) or only active substances (400 mg per tablets in 1.1.1 ratio) are considered to determine the total administered dose?
- Our response: We apologize for the confusion caused by the incomplete explanation. The doses of SH003, 1,200 mg, 2,400 mg, and 4,800 mg indicate active substances. We added following sentence to the study design section: “These doses represent the measurement of active ingredients found in a half of one tablet.”

4) The study is presented as a dose excalation study following the 3+3 design. Does the model follow the Fibonacci’s scheme or other models? The dose levels are determined with the well known dose excalation of 100%-67%-50%- 40%-33%? Or using different percentage steps?). All this aspects are not well defined in the “sample size” paragraph.
- Our response: We initially decided to escalate the dose using modified Fibonacci sequence. Generally, dose escalation using a modified Fibonacci sequence will be the dose first increases by 100% of the preceding dose, and thereafter by 67%, 50%, 40%, and 33% of the preceding doses, as you mentioned. However, since SH003 contains 400 mg of active substances per tablet and is taken three times a day, we could not follow the above ratio. Considering that the maximum recommended starting dose of SH003 is 2,400 mg per day, the third dose was also escalated by 100%, to 4,800 mg. The dose of the present study appears to be escalated to a fixed increment of 100% because it will only be conducted up to 4,800 mg. However, considering the fact that the actual incremental ratios are varied in studies using the modified Fibonacci sequence, we thought that the design of the present study may be called the modified Fibonacci sequence.
We have added following sentences to the study design section: “The dose escalation will follow the modified Fibonacci sequence. The dose will be increased twice by 100% of the preceding dose.”

5) Which are the expected Dose Limiting Toxicities? Which are the expected serious adverse effects? No data come from the preclinical studies session.
- Our response: Thank you for your comments. It enabled us to improve the quality of our manuscript. Based on the toxicity test and the herbology literature, we selected increases in AST, increases in ALT, and diarrhoea as expected dose limiting toxicities. Neutropenia and decreased platelet count, which are major side effects of anticancer drugs, were also selected as expected dose limiting toxicities.
We have added following sentences to the introduction: “Moreover, no toxicity was detected in the efficacy studies. In one toxicity test, hypertrophy of the liver was observed; however, it was deemed to be a reversible change with no toxicological significance. However, an herbology textbook has

mentioned that components of SH003 should be used with caution in patients with diarrhoea.", and to the primary outcome measurement "The expected dose-limiting toxicities include diarrhoea, increases in ALT, and/or AST, febrile neutropenia, and a decreased platelet count"

6) In common Phase I studies on citotoxic or biological drugs Pharmacokinetic's studies are fundamental. In this protocol the Pharmacokinetics investigations are not considered as correctly stated in the discussion part, but this is a clear limitation.
- Our response: We agree with your opinion. So far, we have not been able to conduct pharmacokinetic studies on human subject, however, we will try to conduct pharmacokinetic studies by gathering preclinical pharmacokinetic data steadily.
We have added following sentence to the discussion: "Therefore, it is difficult to collect PK evidence on dose and frequency of administration of herbal medicines.", and changed the sentence "Based on in vivo PK studies currently being performed, further human studies should be conducted." to the "Based on in vivo PK studies on SH003 currently being performed, further human studies will need to be conducted."

Reviewer: 3
1) How have you selected the scaling rule (1200-2400-4800 mg SH003), which is quite different from the classical Fibonacci's one?
- Our response: Thank you for your comments. It enabled us to improve the quality of our manuscript. We initially decided to escalate the dose using modified Fibonacci sequence. Generally, dose escalation using a modified Fibonacci sequence will be the dose first increases by 100% of the preceding dose, and thereafter by 67%, 50%, 40%, and 33% of the preceding doses. However, since SH003 contains 400 mg of active substances per tablet and is taken three times a day, we could not follow the above ratio. Considering that the maximum recommended starting dose of SH003 is 2,400 mg per day, the third dose was also escalated by 100%, to 4,800 mg. The dose of the present study appears to be escalated to a fixed increment of 100% because it will only be conducted up to 4,800 mg. However, considering the fact that the actual incremental ratios are varied in studies using the modified Fibonacci sequence, we thought that the design of the present study may be called the modified Fibonacci sequence.
We have added following sentences to the study design section: "The dose escalation will follow the modified Fibonacci sequence. The dose will be increased twice by 100% of the preceding dose."

2) why the minumin age was equal to 19 yrs and not 18, as intl standard? Korean legal age?
- Our response: Generally, in Korea, a person who is under 19 years of age by law is a minor. Therefore, those who are under the age of 19 have some limitations in doing legal acts alone. Although adults are usually 18 years and older in clinical trials as you mentioned, in some cases, the members of IRB may request to conform to the legal adult age. So, we included "those 19 years-of-age and older" to the inclusion criteria.

3) every solid cancer? every previous chemo regimen? no limitations? in that case, why have you excluded oncohematological neoplasms?
- Our response: We have conducted various preclinical studies on the anticancer efficacy of SH003, but there is no study of hematological neoplasms, so only clinical trials for solid tumors have been proposed and approved.

4) no expected AEs are listed, which type of AEs according to CTCAE are you expecting?
- Our response: Based on the toxicity test and the herbology literature, we selected increases in AST, increases in ALT, and diarrhoea as expected dose limiting toxicities. Neutropenia and decreased platelet count, which are major side effects of anticancer drugs, were also selected as expected dose limiting toxicities.

We have added following sentences to the introduction: “Moreover, no toxicity was detected in the efficacy studies. In one toxicity test, hypertrophy of the liver was observed; however, it was deemed to be a reversible change with no toxicological significance.9 However, an herbology textbook has mentioned that components of SH003 should be used with caution in patients with diarrhoea.”, and to the primary outcome measurement “The expected dose-limiting toxicities include diarrhoea, increases in ALT, and/or AST, febrile neutropenia, and a decreased platelet count.”

5) "MTD will be defined as that dose level at which more than 1 out of 6 patients exhibit dose limiting toxicity during the 4 weeks..." IMHO, this is DLT definition, while MTD one is the last previous dose level, do you agree?
- Our response: We agree with your opinion. We made a mistake in defining MTD. We have changed the sentence “MTD will be defined as that dose level at which more than 1 out of 6 patients exhibit dose limiting toxicity during the 4 weeks of trial period.” into “MTD will be defined as the dose just below the lowest dose level at which more than 1 out of 6 patients exhibit DLT during the 4 weeks of the trial period.”

6) you should report descriptive stats for continuous variables as median(IQR) and for categorical ones as abs/rel frequencies
- Our response: Thank you for your valuable advice. We have changed the sentence “The continuous variables will be displayed as the mean and range, and the categorical variables will be displayed as the numbers.” into “The continuous variables will be displayed as the median and range, and the categorical variables will be displayed as the absolute and relative frequencies.”

VERSION 2 – REVIEW

REVIEWER	Roberto Passera University of Torino-Italy
REVIEW RETURNED	05-Feb-2018

GENERAL COMMENTS	I do believe that the authors correctly solved my previous epidemiological concerns

REVIEWER	Alessandro Comandone Ospedale Humanitas Gradenigo Torino ITALY
REVIEW RETURNED	19-Feb-2018

GENERAL COMMENTS	I read the revised second version of the protocol a single arm , open label, dose escalation Phase I study to evaluate the safety of an herbal medicine SH 003 in patients with solid cancer : a study protocol. In confront with the previous version this second one has considered some suggested major changes. In spite of these changes, some limitations are still evident. 1) A short mention of “efficacy studies “ are reported in the introduction, but more complete and mature data are lacking. 2) As requested, the increase of dose is reported to follow a modified Fibonacci’s scheme , with 100% dose escalation of the three herbal medicines. 3) The aims of the study are better defined than before: determining dose limiting toxicities as adverse effects during the study period is the primary end point. In this version the most common secondary effects are well defined: diarrhoea, haematological and liver toxicities.

	The secondary end point " include AE 's as well as changes in tumor size as assessed by computed CT scan". Volume changes, that is a different way to call objective response, is not a common end point in phase I study. In conclusion the present version can be considered for the pubblication, even if this study remains atypical for a design of Phase I study.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 3

I do believe that the authors correctly solved my previous epidemiological concerns

Our response: Thank you for your comment. Your last comments and suggestion are very helpful for improving out manuscript.

Reviewer: 2

I read the revised second version of the protocol a single arm , open label, dose escalation Phase I study to evaluate the safety of an herbal medicine SH 003 in patients with solid cancer : a study protocol.

In confront with the previous version this second one has considered some suggested major changes.

In spite of these changes, some limitations are still evident.

1) A short mention of "efficacy studies " are reported in the introduction, but more complete and mature data are lacking.

2) As requested, the increase of dose is reported to follow a modified Fibonacci's scheme , with 100% dose escalation of the three herbal medicines.

3) The aims of the study are better defined than before: determining dose limiting toxicities as adverse effects during the study period is the primary end point. In this version the most common secondary effects are well defined: diarrhoea, haematological and liver toxicities.

The secondary end point " include AE's as well as changes in tumor size as assessed by computed CT scan". Volume changes, that is a different way to call objective response, is not a common end point in phase I study.

In conclusion the present version can be considered for the publication, even if this study remains atypical for a design of Phase I study.

Our response: Thank you for your comments. Your comments and suggestion are very helpful for improving our manuscript. We have added additional descriptions and limitations to the 'Introduction' and 'Discussion' about what you pointed out.

The following sentences in ‘Introduction’ section “It has been reported that SH003 suppresses breast cancer growth and metastasis by inducing autophagy and inhibiting STAT3-IL-6 signaling. Moreover, it represses tumour angiogenesis by inhibiting VEGF-induced VEGFR2 activation, and induces apoptosis of prostate cancer cells by inhibiting ERK2-mediated signalling. In vivo xenograft studies have reported that SH003 inhibits tumour growth and metastasis, as well as VEGF-induced tumour angiogenesis without detectable toxicity, and SH003 in combination with doxorubicin has shown a synergistic effect in treating triple-negative breast cancer (TNBC).” have been changed into “It has been reported that SH003 suppresses breast cancer growth and metastasis by inducing autophagy and inhibiting STAT3-IL-6 signaling. SH003 inhibits cell proliferation and induces apoptosis without an effect on normal cell viability. Moreover, it represses tumour angiogenesis by inhibiting VEGF-induced VEGFR2 activation, VEGF-induced phosphorylation of VEGFR2 is blocked by SH003 interrupting VEGF binding to VEGFR2. SH003 induces apoptosis of prostate cancer cells in a dose-dependent manner. This is due to the intracellular mechanisms that SH003 inhibits ERK2-mediated signaling. In vivo xenograft studies have reported that SH003 inhibits tumour growth and metastasis, as well as VEGF-induced tumour angiogenesis without detectable toxicity. SH003 in combination with doxorubicin has shown a synergistic effect in treating triple-negative breast cancer (TNBC). The combinational treatment induces apoptotic cell death and suppresses tumour growth.”

The following sentences have been added into the ‘Discussion’ section. “The pharmacological action of SH003 has not yet been fully elucidated. Thus, preclinical studies for SH003 will also continue to be conducted and the results will be published.”, “Changes in tumour size, that is objective response, are not a common outcome measurement for phase I study, but have been included in the present study, expecting that the results would provide helpful information to plan further studies.”

VERSION 3 – REVIEW

REVIEWER	ALESSANDRO COMANDONE OSPEDALE HUMANITAS GRADENIGO TORINO ITALY
REVIEW RETURNED	17-Mar-2018

GENERAL COMMENTS	Dear Editor, I reviewed for the third time the protocol : “ A single arm , open label , dose escalation Phase I study to evaluate the safety of an Herbal medicine S003 in patients with solid cancer: a study protocol.” Few changes were done at the previous version of the protocol: More data on the activity of the Herbal drug in vitro are available ; better specificity to define the informed consent of the patients. All the other parts of the protocol are unchanged. The limitations of the study had been defined in the first and second review: No pharmacokinetics data, no MTD definition, no full and complete informed consent as stated by Oviedo’s conference acts. I well understand that natural drugs should have a different experimental evaluation in clinical oncology, but is difficult, following such design of the study, to completely define in heavily pre treted patients the real role of S003.

VERSION 3 – AUTHOR RESPONSE

Thank you for your thoughtful advice on the manuscript. All your comments and suggestion has enabled us to improve the quality of our manuscript. We have made some corrections and clarifications in the manuscript. The changes are summarized below.

1) No pharmacokinetics data
Our response: Thank you for your comment. It enabled us to improve the quality of our manuscript. Although it has not yet published, we have conducted pharmacokinetic study on SH003, and we were able to find some clues to pharmacokinetics of SH003. We have added the relevant description to the discussion section, and are planning a clinical pharmacokinetic study of SH003.
We have added the following sentence to the discussion section: “One of the constituents of SH003, Angelica gigas, contains decursin and decursinol angelate, which are characterising compounds of Angelica gigas according to the Korean Pharmacopoeia. Decursin and decursinol angelate have been reported to have anti-tumor activities. Astragalus membranaceus, another constituent herb of SH003, contains calycosin and formononetin. Anti-tumor effect of calycosin and formononetin has also been reported. Therefore, it is reasonable to conduct a pharmacokinetic studies on SH003 using decursin, calycosin, and formononetin as marker compounds. Although it has not yet published, plasma concentrations monitoring of decursin, decursinol angelate, decursinol, calycosin, and formononetin after the administration of SH003 in rat have conducted. In the study, decursin and decursinol angelate showed very low bioavailability, presumably because of the rapid conversion of decursin and decursinol angelate to decursinol in body, and decursinol showed a higher plasma concentration than the other components. Therefore, it suggest that decursinol could be used as a major marker compound in pharmacokinetic study of SH003. Based on these results, a clinical pharmacokinetic study of SH003 is being planned.”

2) no MTD definition
Our response: Thank you for your comment. We have defined the MTD as following in outcome analysis section : “MTD will be defined as the dose just below the lowest dose level at which more than 1 out of 6 patients exhibit DLT during the 4 weeks of the trial period. In the present study, the highest dose among the three dose groups (1200 mg, 2400 mg or 4800 mg per day) with one patient or less experiencing DLT will be determined as the MTD of SH003.”

3) no full and complete informed consent as stated by Oviedo’s conference acts
Our response: Thank you for your comment. We apologize for the insufficient material. We have attached full informed consent form as an additional file.

VERSION 4 – REVIEW

REVIEWER	Alessandro Comandone Ospedale Humanitas Gradenigo, Torino Italy
REVIEW RETURNED	20-Jun-2018

GENERAL COMMENTS	The present study will investigate the tolerability and safety of administering SH003 in patients with solid cancers in advanced stage. As Phase I study the primary outcome is determined by the number of 3 or 4 AEs throughout the study period as measured by the National Cancer Institute scale. Secondary outcome include the AEs, regardless of grade. Inclusion criteria are clear as well as the exclusion ones. All these fidings can lead to define the MTD of SH003 , a mixed herbal extract containing Huang-Qi (Astragalus membranaceus), Dang-Gui (Angelica gigas), and Gua-Lou-Gen (Trichosanthes Kirilowii Maximowicz), which are traditionally used in Korean medicine. SH003 has demonstrated in previous studies some anticancer

	activities as anti angiogenic agent as well as antimetastatic drug as the Authors show. The short period of inclusion of the patients in the study (4 weeks) can be justified as Phase I study, in which therapeutic effects are not considered . Late and remote toxicities are not considered, only acute and dose limiting AE are focused on The Fibonacci's modified scheme 3+3 is an adequate design for this kind of investigation. In this last version the protocol can be accepted for pubblication