Author's response to reviews

Title: Safety and Pharmacokinetics of Novel Selective Vascular Endothelial Growth Factor Receptor-2 Inhibitor YN968D1 in Patients with Advanced Malignancies

Authors:

   Jin Li (fudanlijin@163.com)
   Xinmin Zhao (mizuyi@hotmail.com)
   Lei Chen (chenlei350401@sohu.com)
   Haiyi Guo (guohaiyi-sh@hotmail.com)
   Fangfang Lv (lvff80@yahoo.com)
   Ka Jia (fudanjiaka@163.com)
   Ke Yv (yuk@mail.shcnc.ac.cn)
   Fengqing Wang (wfq@mail.shcnc.ac.cn)
   Chuan Li (chli@mail.shcnc.ac.cn)
   Jun Qian (junqian68@hotmail.com)

Version: 3 Date: 9 August 2010

Author's response to reviews: see over
Dear Editor,

Revised manuscript (# - 4133455573803329)

Thank you again for your patience to allow us to make a thorough revision on the manuscript titled Safety and Pharmacokinetics of Novel Selective Vascular Endothelial Growth Factor Receptor-2 Inhibitor YN968D1 in Patients with Advanced Malignancies.

We would like to thank the reviewers for their constructive and instructive suggestions for our manuscript. We are deeply indebted to the reviewers’ for pointing out some of the shortcomings of our study. We have made a great effort in checking and reviewing all the requested data. The language usage has also been polished and corrections were made to improve readability of our manuscript.

Your assistance and patience has been well received and greatly appreciated.

Thank you again for your time.

Yours sincerely,

Jin Li

Response to Reviewer’s report

Title: Safety and Pharmacokinetics of Novel Selective Vascular Endothelial Growth Factor Receptor-2 Inhibitor YN968D1 in Patients with Advanced Malignancies

Version: 1 Date: 26 May 2010

Reviewer: Mark McKeage

Reviewer’s report:

This is a well written and presented report of an oncology phase I trial that met its objective. The following are suggestions for improvements.

*Major Compulsory Revisions
None

*Minor Essential Revisions
Pg 11 3rd line define abbreviation "HFS"
Reply: "HFS" has been defined in the previous section.

Pg 11 7th line include dose, method of administration and citation for use of glutathione
The use of glutathione was recommended by the nephrologists the authors consulted which stated that it may relieve some of the sign and symptoms but did not provide further citation. The sentence has thus been removed.

*Discretionary Revisions

pg 10 1st paragraph and table 2. Suggest adding graph of dose versus Cmax and AUC to demonstrate non-proportional PK, interindividual variability of PK and PK data for doses other than 750mg.
Reply: All PK data for single-dose and multiple-dose evaluation has been presented in Table 2.
On comparing the 500 mg, 750 and 850 mg single oral administration of YN968D1, when the dose increase with ratio of 1:1.5:1.7, the AUC ratio were 1:1.61:1.95 and C_{max} ratio were 1:1.56:1.86. The exposure increased slightly more than dose proportionally at dose of 850 mg

pg 12 paragraphs 1 and 2. What prior treatment had the responding patients received.
Reply: The first patient (45 years old) with metastatic rectal cancer failed 2 cycles of FOLFOX4 regimen, 4 cycles of liver chemoembolization, and 4 cycles of FOLFIRI regimen prior to entering the study. The second patient (65 years old) with metastatic rectal cancer had 3 cycles of liver chemoembolization, 2 cycles of FOLOX regimen, and refused further chemotherapy. The information has been added to the manuscript.

pg 14 and 15. The claims for greater antitumor activity of study drug compared to others in the class should be more balanced. Qualifications should be added such as these apparent differences possibly being due to patient selection and requiring further prospective comparative study.
Reply: The sentence has been revised.

pg 15 Conclusion. The statement above exploring a twice daily schedule should be removed as it poorly justified as it currently reads. If a specific target concentration is sought, or the drug accumulation that will occur with twice daily is
considered not to be a potential problem, then this should be expanded upon.

Reply:
The statement has been removed.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interest

Title: Safety and Pharmacokinetics of Novel Selective Vascular Endothelial Growth Factor Receptor-2 Inhibitor YN968D1 in Patients with Advanced Malignancies

Version: 1 Date: 30 May 2010

Reviewer: Peter Fong

Reviewer's report
Overall, an interesting and worthy to be reported study of yet another small molecule VEGFR2 inhibitor. Unfortunately, being a first in human study, some of the data reported in the manuscript is slightly patchy. There is no pharmacodynamic correlate within the study but the incidence of hypertension is “reassuring” somewhat. The weight of the discussion leans too much towards response and clinical benefit without some key “traditional” outcomes one expects to be reported from a Phase I study.

Major Compulsory Revisions

Abstract – Results – “The intake of food prior to dosing had no relevant impact on the PK”. This statement as not substantiated in any part of the manuscript. Was this trial designed a priori to be a food effect bioavailability and fed bioequivalence study? If so, please mention it in the methods section, provide details and provide the PK data for apatinib levels in the fasted and fed situation.
Reply:
The effect of food on the pharmacokinetics of YN968D1 was assessed in a randomized open-label, two-way crossover study. A single oral dose of 750-mg was administered to 12 healthy subjects after a 10-h fast in one period (fasted) and after a light meal in the other period (fed). A washout period of 7 days separated YN968D1 dosing between the two treatment periods. The 90% confidence intervals (CIs) for maximum plasma concentration (Cmax) and area under the concentration–time curve (AUC) were within the 80–125% bioequivalence range. The intake of food prior to dosing had no significant effect on single-dose pharmacokinetics.

Confined to the length of this paper, the study on food effect may be not suitable to be described in detail here and may be written independently later. Therefore, we consider deleting the statements which related to the food effect from the original manuscript.

Background – paragraph 2 and 3 – statements of “9 times of inhibition to VEGFR2”, “mildly inhibits c-Kit and c-Src” and “on the basis of encouraging preclinical data” are vague and confusing: Please provide the reference and details of the preclinical data. Is there an update to the AACR proceedings from 2006?
Reply:
The reference and details were extracted from the Apatinib Investigator Brochure which is unpublished and confidential. We have deleted the statements. There is no update to the AACR proceedings from 2006.

Methods -Study design – It is unusual for fever caused by infection during administration of study drug to be excluded from assessment of possible DLT. The statement is ambiguous and suggests that neutropenic fever is also excluded as a DLT. Please clarify if the preclinical studies of apatinib revealed any evidence myelosuppression or pyrexia. Please provide justification for the above.
Reply:
The DLT has been defined correctly in the revised manuscript. There is no preclinical study of apatinib that revealed any evidence of pyrexia.

Methods -Pharmacokinetic analyses – Was PK analyses performed for all dose levels from 250 to 1000mg once daily? The methods section suggests this was not. Please clarify. Was any PK performed for the patients dosed at 250 and 1000mg/day?
Reply:
The study design has been added to the revised manuscript to explain the requirement of dose escalation and PK following the Chinese SFDA guidance. Thus, PK was performed for single-dose 500mg, 750mg and 850mg and multiple-dose at 750mg. One of the patients (last one for dose escalation) in the
850mg dose escalation cohort consented to participate in the 850mg PK analysis.

Could the authors confirm if any pharmacodynamic (PD) studies were undertaken?
Reply:
Pharmacodynamic studies had been undertaken in this study for six patients but the authors had decided to prepare another manuscript independently for later submission.

Results – Dose escalation – it is confusing how many patients were actually treated at their initially assigned dose levels during dose escalation. Please state the number of patients for each dose cohort for both the dose escalation and PK phase. The number of patients treated during the PK analysis aspect was 28 yet 32 patients were assessable for PK analysis, please clarify. Is there any PK data for the 1000mg/day dose level, especially for the 2 patients with DLT? This is important not only because no PD data has been reported, as in the section of Results – PK – a statement was made that there was no relationship between drug related AE, dose and extent of apatinib exposure, yet there is only data for the 750mg/day dose. The final statement of “interpatient variability of apatinib PK does not affect its clinical safety” is therefore not substantiated by the data presented thus far.
Reply:
The study design meeting the SFDA guidance has been added to the Methods section, which has also been reflected in the Result section. PK was not approved by the Fudan University Shanghai Cancer Center Ethic Committee for Clinical Investigation for the 250mg-cohort. There is no PK data for the 1000mg/day dose level for the 2 patients with DLT per SFDA guidance and requirements. The number of patients has been reviewed and corrections have been made. The exact patient number for each dose level has been clarified in the revised manuscript.
19 patients were included in the dose-escalation phase, and one was also enrolled in the 850mg PK cohort. Another additional 27 patients were enrolled for PK analysis. Thus the total number of patients assessable for PK was 28.
Data was insufficient for the analysis of association between drug exposure and drug-related AE due to the high interindividual variability and the limited number of patients. Thus the statement has been removed.

Results – Table 2 -12 patients were treated at dose level 750mg/day but only 11 patients were analysed for the mean PK parameters in Table 3. Please clarify the discrepancy.
Reply:
One patient withdrew consent for the multiple-dose cohort (750mg) after completion of the 750-mg-single-dose assessment. This information has been added as described above.
Results – PK – No results are shown for the 250mg, 500mg, 850mg and 1000mg/day dose cohort, yet it is mentioned that \(c_{\text{max}}\) and AUC increased with dose. I do not believe this conclusion can be reached on the available information presented in the manuscript. The data in Table 2 is only a reflection of the 750mg/day dose cohort.

Reply:
All PK data for the 28 patients has been presented in Table 2.

Results – tumour response – 38 of 47 patients were evaluable for tumour response. Please provide the reasons for the 9 patients who were not. Furthermore, if only 22 out of 30 colorectal and gastric cancer patients were evaluable for response by RECIST, this means that of the total of 47 patients, 8 out of 9 unevaluable patients were either colorectal or gastric cancer patients. The proportion of evaluable to unevaluable colorectal and gastric cancer patients is rather high. Whilst tumour response is not a primary endpoint of a first in human phase I study, the manuscript asserts strongly the “substantial antitumor activity of apatinib and the discussion leans heavily towards response assessment. Although the activity observed is encouraging, it is premature and the authors should reflect this in a balanced manner in the discussion.

Reply:
Patient data has been reviewed. The number of patients has been corrected and the reasons for patients not evaluable for response had been stated in the revised manuscript. There were 46 patients included and 45 were with measurable lesions. Reason for not evaluable were: 4 withdrew consent due to receiving other therapy, one intolerable toxicity, and three were lost to follow-up.
We have removed the statement on response observed specifically among GI patients since there is no difference between cancer types.

Discussion – 2nd paragraph – There is no data submitted thus far to substantiate the statement that the mean half-life was constant over all dose groups. Likewise, there is no PK data to support a dose of 850mg daily. Please provide the supporting data.

Reply:
All PK data for single and multiple dose evaluation have been provided in Table 2.

Discussion – please clarify the statement of “significant interpatient variability with apatinib provided individual data for dose justification”. It is unclear what you are trying to convey, the interpatient dose variability at 750mg is not hugely different to some oncology Phase I trials of small molecule TKI’s. You have demonstrated that there were more dose reductions required at the 850mg/day dose level compared to 750mg/day but there is no collaborative PK data. The statement of “these data indicated no apparent relationship between drug-related toxicity and
plasma............” is not substantiated. The word “justification” has been incorrectly used. It should be dose modification. The author is trying to explain that dose modification to meet individual needs is necessary (in future clinical studies) due to the significant interpatient variability with apatinib. This has been clarified in the revised manuscript.

Discussion - Of the patients who responded, how many were at the 750mg/day dose level vs. 850mg/day? What justification is there for 850mg/d to be the RP2D if there is not an insignificant proportion requiring dose reduction? Responses of patients for each dose level were presented in Table 4. None of the 6 patients enrolled to the 850mg/day dose level experience any DLT. It may be a recommended dose since it is the next lower dose from the MTD (at 1000mg/day).

Minor Essential Revisions

Methods – Study design - It is unclear what the primary and secondary objectives of this phase I trial are. Please outline these clearly.
Reply: The primary and secondary objectives of this phase I trial have been clarified in the Introduction section as below:

Methods - Pharmacokinetic analyses – The authors assert that the apatinib concentrations were determined by “fully validated LC/MS methods”, please insert a reference pertaining to this methodology.
Reply: The validated LC-MS/MS method for quantitative determination of YN968D1 was performed at Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The manuscript is under preparation for submission to the relevant journal.

Results – tumour response – please define “experienced control of the disease”. It would be useful to mention all the tumour types that were associated with a partial response to apatinib (n=7). Similarly in the conclusion, is it not appropriate to state there was striking activity in a broad range of solid tumours unless detailed. The tumor types that were associated with a PR to apatinib had been stated in the manuscript. Among the 7 patients who achieved PR, one was diagnosed with GIST, one cancer of unknown primary, one renal cell carcinoma, one gastric cancer, and 3 colon cancer.

Discussion – paragraph 5 – “patients who experienced dose reduction could keep control of disease to 5.4 months”. This is unclear, does this mean that only patients who needed a dose reduction had disease stabilisation of 5-6 months or was this also observed in those who did not need a dose reduction for toxicity reasons. It is
probably reflected also by the biology of the tumour rather than an ability to
tolerate apatinib. Other explanations for example resistance to small molecule
VEGFR inhibiting TKI’s exist and probably deserve to be mentioned.

It has been clarified in the revised manuscript that SD for 5 or 6 months was noted
among patients with or without dose reduction, suggesting a lower/tolerable dose
may be able to achieve disease control than the predefined 8 weeks.

Discussion – 7th paragraph – IC50 units missing. Please state the duration of PR
of the GIST patient(s). Note Table 1 states there is only 1 GIST patient but
discussion states 2 patients.

Reply:
The IC50 unit has been added. The duration of PR for the GIST patient is 24
months and the number of GIST patient has been corrected to ONE GIST patient.

Please cite the 2009 GI ASCO proceedings correctly.

Reply:
The abstract has been cited correctly in the revised manuscript.

Conclusions – It is not appropriate to state there was striking activity in a broad
range of solid tumours.

Reply:
The sentence has been revised.

Table 4 – it may read better if the Grade 1/2 and 3/4 adverse events were
displayed as columns rather than rows.

Reply:
The table for adverse events has been revised as recommended and named
Table 3.

Discretionary Revisions

Study design – please clarify how the first dose level of 250mg/day was selected.

Reply:
The selection of the first dose level of 250mg/day has been clarified and stated in
the revised manuscript. The safe starting dose was determined according to
SFDA recommendation that corresponded to one-fourth of the MTD in dogs.

Poor choice of reference (ref 1) for overview of VEGFR family of proteins.

Reply:
More appropriate references for overview of the VEGFR family of proteins have
been provided.

Figure 2 is not very convincing for a PR on the resolution of the image submitted.

Reply:
The response has been re-evaluated by radiologist and was confirmed to meet the response criteria of PR. The lesion shown was among one of the total target lesions which has a better image resolution.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published.

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests