PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Study Protocol – A Randomized, Double-Blind, Parallel Group, Placebo-Controlled Study to Evaluate the Analgesic Efficacy and Safety of VVZ-149 Injections for Post-Operative Pain Following Laparoscopic Colorectal Surgery
AUTHORS	Nedeljkovic, Srdjan; Correll, Darin; Bao, Xiaodong; Zamor, Natacha; Zeballos, Jose; Zhang, Yi; Young, Mark; Ledley, Johanna; Sorace, Jessica; Eng, Kristen; Hamsher, Carlyle; Maniam, Rajivan; Chin, Jonathan; Tsui, Becky; Cho, Sunyoung; Lee, Doo

VERSION 1 - REVIEW

REVIEWER	Hyun Kang M.D., Ph.D., M.P.H. Chung-Ang University, College of Medicine
REVIEW RETURNED	09-Feb-2016

GENERAL COMMENTS	This is a nicely written manuscript with good use of English, concrete study design and appropriate statistical analysis that worth to be published. But I have some minor concerns to this manuscript. 1. Introduction section 1) Line 9 to 12 in 5th paragraph - There is no reference regarding Phase 1 study of VVZ-149. If any, it would be valuable to make a reference: abstract, supplement, or electronic citation is possible if it is not a journal article according to the instruction about BMJ reference style. 2. Methods and study design section (1) Baseline assessment, treatment phase, and post-treatment follow up 1) Line 5 to 6 in 2nd paragraph: regarding potential confounders to affect pain reporting and opioid use - Although you highlighted the assessment of the potential confounders of analgesic response (anxiety, depression, and catastrophizing behaviors), there is no description of statistical analysis regarding these confounders. 2) “Expectations of Treatment” questionnaire - Please make a reference. 3) Assessment of respiratory depression - Please make a reference or present in detail. In addition, there is no description regarding the case of respiratory depression which can be clinically significant. 4) Line 8 in 3rd paragraph - It does not seem clear to describe the time to evaluate a pre-dose pain intensity score as “when regaining alertness”. It needs to be clarified using the RASS or Aldrete score, etc. In addition, time interval from the end of surgery until patients become alert enough

	to report their pain intensity as a score obviously may be extremely prolonged and this leads to the start delay of study medication. Thus, it would be more rigorous study protocol with description about your strategy in this case. 5) Line 1 to 3 in 7th paragraph - The description "… continuous pulse oxymetry for up to 12 hours post-dosing of VVZ-149 or during IV PCA use up to 24 hours" conflicts with the blindness. 6) Line 3 in 9th paragraph/ Line 11 in 2nd paragraph in the Outcome section - The description "post-operative nausea and vomiting scale" is ambiguous. According to study protocol in both your manuscript and clinicaltrials.gov, the evaluation issue of PONV is the incidence. 7) Line 5 in 9th paragraph - "… obtained at several time points …" : Please describe the time points in detail. - "Blood samples for pharmacokinetic analysis are also obtained": Please describe the time points of blood samples for pharmacokinetic analysis. 8) 10th paragraph - Please make a reference of "Perception of Treatment" questionnaire. In addition, there no description of statistical methods regarding two planned evaluations: the robustness of blinding; correlation between treatment expectation/overall satisfaction and treatment outcomes. (2) Randomization and blinding 1) 3rd paragraph - There is no description about your strategy after unblinding and following discontinuation of the study medication. (3) Anesthesia protocol 1) Opioids administered during surgery - Several opioids are administered intraoperatively although only fentanyl is used within 1 hour of surgery completion. Moreover, there is no description about concrete administration dosages except for upper limits. These may affect the pre-dose pain intensity which is decisive in determining whether patient is eligible or not. 2) No more than 20 ml of 1% lidodcaine anesthetic wound infiltration may be given. - Wound infiltration with even small dose of lidocaine may have an influence on pre-dose pain intensity. 3) At the discretion of the anesthesiologist, subjects may receive standard doses of intra-operative prophylactic anti-emetics (dexamethasone, haloperidol, and ondansetron). - Since the incidence of PONV is secondary outcome as safety data, several anti-emetics administered at anesthesiologist's discretion can affect the outcome. In addition, there is no strategy for PONV, which should be described in your protocol. 4) … fentanyl up to 30 μg/kg… - Please unify the unit of fentanyl dosage, mcg vs. μg. 3. Outcomes section 1) Line 2 in 2nd paragraph: "…multiple time segments…" - It would be more valuable to describe as you mentioned at clinicaltrials.gov: 0-2, 2-4, 6-8, 8-12, 12-16, and 16-24 hours post-dosing of study drug. 4. Statistical methods section (1) Analysis of secondary endpoints 1) Line 2: "… intensity, and number and duration of PONV …" - Please clarify the intensity for what.

	- According to the protocol in your manuscript and clinicaltrials.gov, there was no description about the duration of PONV.

REVIEWER	Nina Klemann Copenhagen Prostate Cancer Center, Rigshospitalet, Denmark.
REVIEW RETURNED	15-Apr-2016

GENERAL COMMENTS	I have doubts about the study design: It is stated, that the study aims to investigate whether the study drug is safe and efficient in relieving post-operative pain compared to opiod analgesia alone, however both groups (study drug vs placebo) have opiods available in a PCA-setting during the entire time in the PACU. Furthermore I find it problematic, that the group randomized to receive the study drug, will receive a continuous infusion for eight hours, whereas the control group will receive placebo in a similar manner. I think that there is a large bias in that the continuous infusion of analgesia will inevitably lead to a lower pain score than that experienced by the placebo-group that will only have the possibility of 3 PCAs of hydromorphone per hour. Also, since patients randomized to placebo will almost certainly experience more pain than the group receiving a continuous infusion of an analgesic, the secondary endpoints such as time to first rescue dose and total number of rescue doses will almost certainly differ largely between groups and seem superfluous.

REVIEWER	Andrew Day Surrey and Sussex Healthcare NHS Trust, UK
REVIEW RETURNED	02-May-2016

GENERAL COMMENTS	This is a well thought out and carefully designed study investigating an important issue in laparoscopic colorectal surgery. One potential confounder, given the cohort size, may be the distribution of right and left resections that may exist between the groups following randomisation. The authors have identified this as a potential limitation with the study. Otherwise, I look forward to reading the final analysis with interest.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name

Hyun Kang M.D., Ph.D., M.P.H.

Institution and Country

Chung-Ang University, College of Medicine

Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below
This is a nicely written manuscript with good use of English, concrete study design and appropriate

statistical analysis that worth to be published.
But I have some minor concerns to this manuscript.

1. Introduction section
1) Line 9 to 12 in 5th paragraph
- There is no reference regarding Phase 1 study of VVZ-149. If any, it would be valuable to make a reference: abstract, supplement, or electronic citation is possible if it is not a journal article according to the instruction about BMJ reference style.

Response: A reference has now been cited regarding the Phase 1 study of VVZ-149. This information has been submitted to the FDA as is part of the IND application. Please note that citation (21) has been added to the reference list.

2. Methods and study design section
(1) Baseline assessment, treatment phase, and post-treatment follow up
1) Line 5 to 6 in 2nd paragraph: regarding potential confounders to affect pain reporting and opioid use
- Although you highlighted the assessment of the potential confounders of analgesic response (anxiety, depression, and catastrophizing behaviors), there is no description of statistical analysis regarding these confounders.

Response: Analysis of confounders of analgesic response is being done as part of assessment of our overall outcome measures. We have added a section under the “Statistical Methods” part of the manuscript, entitled “Analysis of Potential Confounders”. We intend to use the ANCOVA model with likelihood ratio testing to assess for the impact of potential confounders.

2) “Expectations of Treatment” questionnaire
- Please make a reference.

Response: A reference has been added pertaining to the “Expectations of Treatment” questionnaire and its role as a possible confounder. This is now citation (28), which has been added to the reference list.

3) Assessment of respiratory depression
- Please make a reference or present in detail. In addition, there is no description regarding the case of respiratory depression which can be clinically significant.

Response: A reference has been added regarding assessment of respiratory depression. We have indicated that a level of respiratory rate <8/min and an oxygen saturation <90% are considered clinically significant. Citation (29) has been added to the list of references.

4) Line 8 in 3rd paragraph
- It does not seem clear to describe the time to evaluate a pre-dose pain intensity score as “when regaining alertness”. It needs to be clarified using the RASS or Aldrete score, etc. In addition, time interval from the end of surgery until patients become alert enough to report their pain intensity as a score obviously may be extremely prolonged and this leads to the start delay of study medication. Thus, it would be more rigorous study protocol with description about your strategy in this case.

Response: The manuscript has been revised to indicate that the level of alertness must be a RASS score of -1 or greater before the study drug may be started. Our standard policy for starting analgesics allows for the patient to be drowsy but with sustained awakening > 10 seconds upon arousal to voice. We have also indicated that this assessment must occur within one hour of

emergence from anesthesia. Patients who do not fulfill this criterion are ineligible to participate further in the study.

5) Line 1 to 3 in 7th paragraph
- The description "… continuous pulse oxymetry for up to 12 hours post-dosing of VVZ-149 or during IV PCA use up to 24 hours" conflicts with the blindness.

Response: Since both groups in the study are receiving IV hydromorphone PCA after surgery, regardless of treatment arm, both groups will be monitored with continuous pulse oximetry. Continuous monitoring is based on the use of PCA with opioids, so that both groups (VVZ-149 + opioids and placebo + opioids) will be monitored. Therefore, blinding will not be compromised. The text has been corrected in this section o page 6.

6) Line 3 in 9th paragraph/ Line 11 in 2nd paragraph in the Outcome section
- The description "post-operative nausea and vomiting scale" is ambiguous. According to study protocol in both your manuscript and clinicaltrials.gov, the evaluation issue of PONV is the incidence.

Response: The PONV scale allows us to examine the incidence and the clinical importance of post-operative nausea and vomiting. For both the treatment arm and the placebo arm, we are interested in understanding if subjects after this type of bowel surgery have just one brief incident of nausea, or if these symptoms are more frequent or constant. The text within the manuscript has been revised to clarify the type of data being collected by the PONV scale.

7) Line 5 in 9th paragraph
- "… obtained at several time points …" : Please describe the time points in detail.
- "Blood samples for pharmacokinetic analysis are also obtained": Please describe the time points of blood samples for pharmacokinetic analysis.

Response: The time points for PK analysis are now listed in the revised manuscript submission.

8) 10th paragraph
- Please make a reference of "Perception of Treatment" questionnaire. In addition, there no description of statistical methods regarding two planned evaluations: the robustness of blinding; correlation between treatment expectation/overall satisfaction and treatment outcomes.

Response: Every effort will be made to maintain blinding of subjects and evaluators. All baseline and follow up interviews will be conducted by research assistants who will remain blinded throughout the duration of the study. Patients and investigators will be asked to guess which treatment the patient actually received and to provide a reason if they believe they know which treatment was received. Treatment guesses will be compared to actual treatment assignments to assess the adequacy of blinding in this study protocol.

Specific instances of un-blinding will be recorded and at the interim analysis (60 subjects), the statistician will monitor treatment guesses to diagnose problems with the blinding procedure. Using Fisher's exact test, we will formally compare treatment guesses between arms at interim analysis and when the study results are reported.

Citation (32) has been added to the list of references. The statistical plan has been clarified in the "Statistical Methods" section of the manuscript for correlation between overall satisfaction and results under the "Analysis of Potential Confounders." For evaluation of the robustness of blinding, we will use Fisher's exact test as indicated above.

(2) Randomization and blinding
1) 3rd paragraph
- There is no description about your strategy after unblinding and following discontinuation of the study medication.

Response: A notation has been added regarding strategy after unblinding. Subjects will receive opioid analgesics as needed at the discretion of their clinician.

(3) Anesthesia protocol
1) Opioids administered during surgery
- Several opioids are administered intraoperatively although only fentanyl is used within 1 hour of surgery completion. Moreover, there is no description about concrete administration dosages except for upper limits. These may affect the pre-dose pain intensity which is decisive in determining whether patient is eligible or not.

Response: Opioid doses will be determined by the clinical discretion of the anesthesiologist based on subjects' surgical response and hemodynamic parameters while under anesthesia. The amount of opioid administered will be converted to a total morphine equivalent dose and will be considered in evaluating results in the analysis of secondary outcomes.

2) No more than 20 ml of 1% lidodcaine anesthetic wound infiltration may be given.
- Wound infiltration with even small dose of lidocaine may have an influence on pre-dose pain intensity.

Response: Lidocaine infiltration is permitted to reduce the possibility that a subject will emerge from anesthesia with intense pain, which is concerning from an ethical perspective. Therefore, we have allowed a small amount of lidocaine to provide analgesia in the period of time between emergence from anesthesia and the start of drug administration in the PACU. Only subjects who have a pain intensity ≥4 on a 10-point scale are considered eligible for enrollment. Since both the placebo and study drug group will be treated in the same manner, the role of lidocaine on postoperative pain scores will affect both treatment arms similarly. The overall results of the study over the ensuing 8 hours (and subsequent assessments up to 24 hours) should be minimally affected, since the duration of action of lidocaine is typically one hour or less.

3) At the discretion of the anesthesiologist, subjects may receive standard doses of intra-operative prophylactic anti-emetics (dexamethasone, haloperidol, and ondansetron).
- Since the incidence of PONV is secondary outcome as safety data, several anti-emetics administered at anesthesiologist's discretion can affect the outcome. In addition, there is no strategy for PONV, which should be described in your protocol.

Response: We will be analyzing the use of these anti-emetics between the control and active drug study groups to determine whether there are differences between groups in the incidence of nausea and vomiting. Since we expect that PONV will be strongly related to opioid consumption after surgery, we will analyze this covariate in our assessment of secondary outcomes. Subjects may receive the medications listed postoperatively for PONV, as needed, and the use of these medications will be considered in assessing between-group outcomes.

4) … fentanyl up to 30 μg/kg…
- Please unify the unit of fentanyl dosage, mcg vs. μg.

Response: This has been unified throughout the manuscript.

3. Outcomes section
1) Line 2 in 2nd paragraph: "…multiple time segments…"
- It would be more valuable to describe as you mentioned at clinicaltrials.gov: 0-2, 2-4, 6-8, 8-12, 12-16, and 16-24 hours post-dosing of study drug.

Response: This has been corrected.

4. Statistical methods section
(1) Analysis of secondary endpoints
1) Line 2: "… intensity, and number and duration of PONV …"
- Please clarify the intensity for what.

Response: This has been clarified. The intensity of PONV refers to the number of instances and the duration of PONV. These data are captures on the PONV questionnaire, which assessed the overall clinical importance of PONV.

- According to the protocol in your manuscript and clinicaltrials.gov, there was no description about the duration of PONV.

Response: The duration of PONV is captured in the PONV questionnaire and is a factor in determining the clinical importance of this adverse effect.

Reviewer: 2

Reviewer Name

Nina Klemann

Institution and Country

Copenhagen Prostate Cancer Center, Rigshospitalet, Denmark.

Please state any competing interests or state 'None declared':
None declared.

Please leave your comments for the authors below
I have doubts about the study design: It is stated, that the study aims to investigate whether the study drug is safe and efficient in relieving post-operative pain compared to opioid analgesia alone, however both groups (study drug vs placebo) have opioids available in a PCA-setting during the entire time in the PACU.

Response: Because the clinical efficacy of this investigational product is not known, IV opioids have been made available to subjects for ethical purposes, in the event that the drug proves inefficacious. We will assess efficacy by evaluating both NRS pain scores and by assessing whether subjects who received the study drug had lower consumption of opioid during the duration of the drug's action. Either of these outcomes would support efficacy of the investigational product.

Furthermore I find it problematic, that the group randomized to receive the study drug, will receive a continuous infusion for eight hours, whereas the control group will receive placebo in a similar manner. I think that there is a large bias in that the continuous infusion of analgesia will inevitably lead to a lower pain score than that experienced by the placebo-group that will only have the possibility of

3 PCAs of hydromorphone per hour.

Response: Both groups (placebo and study drug) will receive the same duration of a continuous infusion and will be blinded to the contents of the infusion. Because we do not know how effective the study drug will prove to be, it is uncertain that one group will have more or less opioid delivery in addition to the continuous infusion. To assess for bias, we will be administering a “perception of treatment” questionnaire to both study staff and subjects. At interim analysis (60 completed subjects) we will analyze the strength of our blinding. The PCA group and the study drug group will both have an opportunity to use PCA every 6 minutes, in addition to receiving supplemental PCA dosing, up to 3 doses additionally each hour.

Also, since patients randomized to placebo will almost certainly experience more pain than the group receiving a continuous infusion of an analgesic, the secondary endpoints such as time to first rescue dose and total number of rescue doses will almost certainly differ largely between groups and seem superfluous.

Response: We are uncertain as to the actual efficacy of the drug, so we do not know whether the secondary endpoints will differ. Indeed, we are evaluating these secondary endpoints to assess drug efficacy in terms of its onset and duration of action. We will report our findings in the results section of our manuscript once the data has been analyzed.

Reviewer: 3

Reviewer Name

Andrew Day

Institution and Country

Surrey and Sussex Healthcare NHS Trust, UK

Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below
This is a well thought out and carefully designed study investigating an important issue in laparoscopic colorectal surgery.

One potential confounder, given the cohort size, may be the distribution of right and left resections that may exist between the groups following randomisation. The authors have identified this as a potential limitation with the study.

Otherwise, I look forward to reading the final analysis with interest.

Response: Thank you for your review, and we similarly look forward to analyzing the data once it has been collected. We will be obtaining data on the nature and type of surgery for all subjects, including the number of right or left resections.

VERSION 2 – REVIEW

REVIEWER	Nina Klemann Copenhagen Unniversity Hospital, Rigshospitalet. University of Copenhagen, Denmark.
REVIEW RETURNED	29-Jun-2016

GENERAL COMMENTS	Since patients "who have clinically important renal or hepatic impairment " (p 6, line 33) are excluded, it could be mentioned how the study drug is metabolized.