PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Multicentric randomized study of H. pylori eradication and pepsinogen testing for prevention of gastric cancer mortality: the GISTAR study
AUTHORS	Leja, Marcis; Park, Jin Young; Murillo, Raul; Liepniece-Karele, Inta; Isajevs, Sergejs; Kikuste, Ilze; Rudzite, Dace; Krike, Petra; Parshutin, Sergei; Polaka, Inese; Kirsners, Arnis; Santare, Daiga; Folkmanis, Valdis; Daugule, Ilva; Plummer, Martyn; Herrero, Rolando

VERSION 1 - REVIEW

REVIEWER	M. Constanza Camargo National Cancer Institute, United States
REVIEW RETURNED	27-Apr-2017

GENERAL COMMENTS	The authors present the GISTAR study, a European multicenter randomized trial of H. pylori eradication and pepsinogen testing. The study addresses the suggestive but limited evidence for H. pylori eradication to prevent gastric cancer. Authors should be commended for establishing this important research initiative. Major points that deserve more attention are as follows: 1. Main results of the pilot phase should be presented 2. Please provide additional information on the following study design issues: • Community outreach strategies and target population(s) • Specific methods to equalize proportions (i.e., 50:50) of men and women among study participants • Number and location of recruitment centers • Recruitment goal in Latvia, and if possible, in other potential participating countries • Full list of biospecimens and their collection conditions and volume/amount • For pathology evaluations, the number of pathologists involved in the trial • Selection criteria and sample size for the subgroup of participants who will have eradication verified • Selection criteria for the 200 samples to be tested for antibiotic susceptibility • Data to be collected during the follow-up Minor comments: 1. Abstract: location of the study should be mentioned 2. According to the Introduction, gastric cancer incidence rates in

	Belarus are 42.1/100,000 for men and 17.2 for women. In the Methods, however, lower figures are cited. Please reconcile 3. A reference should be cited for the statement that H. pylori resistance in Latvia is low

REVIEWER	Manon Spaander Erasmus University Medical Center, Rotterdam the Netherlands There might be a conflict of interest for reviewing this study proposal, because our institute is involved.
REVIEW RETURNED	04-May-2017

GENERAL COMMENTS	Nice protocol and important research question to address. See the attached file for some suggestions.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
M. Constanza Camargo
National Cancer Institute, United States

1. Main results of the pilot phase should be presented

Authors' response

While we agree with the reviewer that the pilot study data will provide additional information on the research questions, our intention to submit this manuscript is to present the main study protocol, which has different aims from the pilot study. It is our understanding that BMJ Open considers publication of study protocols that report planned or ongoing research. Although we have mentioned in the manuscript that the pilot study has preceded the main trial, we feel important to concentrate on the main study protocol. The extensive data collected from the pilot study will be presented separately in several publications after thoroughly and carefully planned analyses.

2. Please provide additional information on the following study design issues:

2.1. Community outreach strategies and target population(s)

Authors' response

We have now clarified this under the 'Participants' subsection in the Methods and analysis section. The following paragraph has been added on page 8:

The study participants will be contacted by phone and invitation mails through lists that we obtain from the general practitioners (GPs), local primary care medical centres, and national medical registration databases, as appropriate, in different locations of the potential recruitment centres.
2.2. Specific methods to equalize proportions (i.e., 50:50) of men and women among study participants

Authors' response

This has been added under the 'Participants' subsection in the Methods and analysis section (page 8)

and it reads as below:

We will pay particular attention to keep the gender balance during recruitment, ensuring at least 50% of the participants are men. To achieve this, we will invite men in priority by direct telephone calls and invitation mails while we accept participation of women in case they are the family members of the invited men or express their interest in participating in the study by contacting the study team.

2.3. Number and location of recruitment centers

Authors' response

We have now updated the text on page 7 with the most recent information of the study locations and it reads as below:

The enrolment has been initiated in three study centers in Latvia: Tukums, Dobele and Rezekne (Caucasian population), with the potential expansion to other locations

2.4. Recruitment goal in Latvia, and if possible, in other potential participating countries

Authors' response

The recruitment goal for the GISTAR main study is to include at least 30,000 participants to satisfy the sample size calculation. This goal is now explained in the main text on pages 7 and 8 as below:

Recruitment centres will be set up reflecting the study requirements. One recruitment centre is expected to randomise 3000 study participants, although in locations with smaller number of inhabitants, fewer than 3000 participants are acceptable. Based on the sample size calculation (see below) at least 10 centres, each recruiting 3000 study participants would be required.

2.5. Full list of biospecimens and their collection conditions and volume/amount

Authors' response

According to the GISTAR protocol, we are collecting the following biological materials:

• EDTA full-blood for DNA extraction (2 x 5 ml); to be frozen and stored at -70°C, transported on dry ice). 1 tube of 10 ml will be allowed as an alternative
• Plasma – 2 aliquots 0.5 ml each, to be frozen within 20 min. after retrieval, and stored at -70°C, transported on dry ice)
• Plasma – 5 aliquots 0.1 ml each, to be frozen within 20 min. after retrieval, and stored at -70°C, transported on dry ice)
• Serum – 5 aliquots 0.1 ml each, to be frozen within 20 min. after retrieval, and stored at -70°C, transported on dry ice)
• Feces for FIT – to be collected in specially designed test-tubes, 1 fecal sample, to be analyzed within 7 days after sampling, high-temperatures to be avoided
• Fecal sample for microbiota– to be stored refrigerated, frozen within 24 hours from material collection
• Standard endoscopy biopsies – according to the pathology protocol (SOP)
• gastric biopsy for microbiota – to be placed in the refrigerator immediately after sampling, frozen at -20°C at least by the end of the working day; for a period exceeding 1 week to be stored at -70°C
In addition, we are planning to collect breath samples for which a detailed protocol will be developed.
In the mauscript, we have now listed all these materials, however without information on the quantity

because the collection may vary in differentt study locations due to various local practice and local requirements. This now reads on under the ‘intervention’ subsection as below:

Biological materials including serum, plasma, DNA, as well as stool and biopsies for microbiota analysis will be collected from different groups of participants for biobanking. Plasma/serum samples will be processed immediately after being obtained, stored and transported at -70°C temperature. These materials will provide the unique opportunity to perform ancillary studies including, but not limited to the following: searching for new biomarkers; and analysing the impact of wide antibiotic use and presence of precancerous lesions on gut microbiome.

2.6. For pathology evaluations, the number of pathologists involved in the trial

Authors’ response

This information has now been added to the ‘Interventions’ subsection on page 9 and it reads as below:

Histological assessment of the biopsies collected from the stomach will be independently performed by two experienced pathologists; in the case of discrepant results, the particular slides will be reviewed together to reach consensus.

2.7. Selection criteria and sample size for the subgroup of participants who will have eradication verified

Authors’ response

To verify the effectiveness of the treatment, we plan to invite 100 to 150 participants who receive eradication therapy from the study centers in Latvia and other centers where resistance patterns are expected to be different based on the available epidemiological data. The following has been added on page 10.

The effectiveness of H. pylori eradication will be verified in a subgroup of participants (n=100-150) from the study centers in Latvia and other centers where resistance patterns are expected to be different based on the available epidemiological data, by using UBT 6-24 months after the treatment.

2.8. Selection criteria for the 200 samples to be tested for antibiotic susceptibility

Authors’ response

In the pilot study, participants with altered biomarkers (i.e. decreased levels of pepsinogens verified either by a latex-agglutination method or ELISA or Gastrin-17) were referred for upper endoscopy in addition to a proportion of participants with normal biomarkers to assess the potential verification bias. Antibiotic susceptibility will be tested using the pilot study data from approximately 200 upper endoscopy referrals with evidence of H. pylori in antral biopsies as described below (page 10).

The susceptibility of H. pylori to commonly used antibiotics in the eradication therapies will be investigated using the pilot study data from approximately 200 upper endoscopy referrals with evidence of H. pylori in antral biopsies (proportion of individuals with altered biomarker results, and another proportion – with normal biomarkers).

2.9. Data to be collected during the follow-up

Authors' response

We have further explained the follow-up data collection on page 10 and it now reads as below:

Particular attention will be given to collect detailed information on potentially H. pylori related morbidity and mortality. Whenever possible, we will invite the participants to the study centres to obtain follow-up data including demographic information, socio-economic status, physical examination as well as biological samples (plasma, serum and stool samples and gastric biopsies for for microbiome testing). The new protocol will be developed to update the follow-up data collection. A record linkage will also be made to the national Cancer and Mortality Registry database to ascertain cases of and deaths from gastric cancer.

3. Minor comments:

3.1. Abstract: location of the study should be mentioned

Authors' response

The GISTAR study aims to investigate whether H. pylori eradication combined with non-invasive screening of precancerous lesions by pepsinogen tests reduces mortality from gastric cancer in Europe. The study has been initiated in Latvia, one of the high gastric cancer risk areas in Europe. We aim to expand the GISTAR study in other areas with high risk, such as Eastern European regions including Belarus, Russia, Latvia and Ukraine. We have included our target areas for the study in the abstract.

3.2. According to the Introduction, gastric cancer incidence rates in Belarus are 42.1/100,000 for men and 17.2 for women. In the Methods, however, lower figures are cited. Please reconcile

Authors' response

The presented ASR of 42.1/100,000 for men in Belarus was for gastric cancer incidence (source: Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013;49(6):1374-403), to describe general gastric cancer burden in the region while the lower figure presented in the Methods section which is 20.2/100,000 for men is for gastric cancer mortality in 2011 as our study endpoint is gastric cancer mortality (source: World Health Organization Cancer mortality database, available from http://www-dep.iarc.fr/WHOdb/WHOdb.htm).

3.3. A reference should be cited for the statement that H. pylori resistance in Latvia is low

Authors' response

The relevant reference has been added on page 9 (reference number 21: Kupcinskas J, Leja M. Management of Helicobacter pylori-related diseases in the Baltic States. Digestive diseases 2014;32(3):295-301).

Reviewer: 2
Manon Spaander
Erasmus University Medical Center, Rotterdam the Netherlands

¬ Objectives: The study could be more of value when also taking cost-effectiveness into account.

Especially since in the introduction the authors described this as a shortcoming of previous studies

Authors' response

We acknowledge that cost effectiveness of a public health strategy is an important issue for the decision-making process. Cost-effectiveness analysis has not been described in the initial protocol, however, the possibility of running such studies has been considered.

The following additional text has been included to the manuscript:

It is expected that the obtained data will allow running cost-effectiveness ancillary studies on mass-eradication of H.pylori by considering the costs of the adverse effects as well as on endoscopic surveillance of patients with gastric precancerous lesions in European countries with a relatively high-risk.

¬ Study design:
a. It is not clearly stated if this study will be population based. Please define if this is the case. When striving for a population based study, current design is not sufficient and following suggestions have to be taken into consideration:
i. When offering FIT CRC screening as an incentive for participation, the participation rates in this study could not be generalized to the general population, and therefore the study design is not population-based. This would attract a certain type of person with increased risk for gastric cancer e.g. above 50 and who could be more interested in their health status. Moreover previous cost effectiveness studies primarily suggest eradication at a relatively early stage in life since infection is generally acquired during childhood.
ii. The authors should not aim at making an extra effort on inviting men or women to balance gender ratio. This will not lead to accurate population based participation rates.

Authors' response

To achieve the main objective of the GISTAR study, we invite participants in Latvia from the general population through the lists of the GPs where everyone in the area is registered. While we acknowledge provision of FIT as an incentive for study participation may influence the generalisability of the study, we would like to emphasise that the participants are not self-selected, which is essential when we evaluate the public health intervention strategy. With regard to the latter point, however, we are aware that the extra effort to keep the gender balance in the study will influence the generalizability of the study results, as the reviewer correctly pointed out. However, the internal validity of the study results must be the primary objective and ensuring the gender balance is essential to keep the study power as we planned due to substantially higher incidence of gastric cancer in men. We have now acknowledged this as one of our limitations of the study and elaborated it more in detail in the Discussion section (page 14). This reads as below:

Although the study participants come from the general population, we acknowledge that our extra effort to balance the male and female ratio to ensure sufficient study power to answer the research questions will influence the generalisability of the study results.

b. Susceptibility of H. pylori treatment will be investigated in a subgroup of participants. It is unclear from what this subgroup holds. Furthermore, since influence of H. pylori eradication on gastric cancer mortality is the primary outcome of this study, it is to consider to evaluate all eradication treatments instead of subgroups. Also determining successfull eradication is advised according to the Kyoto concensus.

Authors' response

We acknowledge that testing for the eradication success in clinical practice is recommended by the Kyoto consensus However, our study aims to evaluate this as part of a public health strategy and we consider that confirmation of eradication would not be included in a mass screen and treat program. Thus, confirmation of eradication in each individual is beyond the scope of the current study aims, as approved by the corresponding ethical committees However, we agree with the reviewer that it is important to verify the eradication success in the study cohort as a group to make sure the study regimens are effective. That is why we plan to do it in subgroups across different regions which may have different resistance patterns. We have now added additional texts to the "Interventions" section for clarity (page 10).

The effectiveness of H. pylori eradication will be verified in a subgroup of participants (n=100-150) from the study centers in Latvia and other centers where resistance patterns are expected to be different based on the available epidemiological data, by using UBT 6-24 months after the treatment.

c. Establishing infection: IgG does not specify past or current infection, using an IgG test to measure infection and then prove eradication by urease breath test may produce false positive results for successful eradication. Possibly positive serologic tests can be followed up by a urease breath test to confront this issue.

Authors' response

We acknowledge the above drawbacks of serology for detecting the presence of H. pylori. However, serology was initially chosen because of its acceptability, feasibility and efficiency in use in a large population to be adapted as part of a public health intervention strategy. Nonetheless, the preliminary results from the pilot study (manuscript in preparation) have indicated false positivity of serology as correctly pointed out by the reviewer. Due to low specificity revealed in the pilot study the investigators have recently decided that serology will not be used as the primary test. We have included this now on page 9 as below:

For H. pylori infection testing IgG group antibodies by ELISA (Biohit, Plc., Finland) was initially planned, however based on the preliminary result from the pilot study which indicated false positivity of serology, 13C-urea breath test (UBT) is decided to be used for confirmation of the infection.

d. Pepsinogen test, both groups get tested for the pepsinogen test but no follow up is required in the non H. Pylori group, neither endoscopic or long term. This might also be a mistake in the figure.

Authors' response

Only the interventional group will be tested for pepsinogens and endoscopic procedures will follow when the level is below the pre-defined cut off values.

For the follow-up, both of the groups will be followed at 5 year intervals for 15 years as indicated in the Methods section (also indicated in Figure 1). Since the endoscopic procedures are conducted only in the interventional group, endoscopic follow-up will be offered only for those in whom precancerous lesions will be identified according to the MAPS guidelines.

e. The results of the pilot study is repeatedly mentioned however these results are not described in

this study it is also unclear if this pilot study has finished or not.

Authors' response

The recruitment of the pilot study has been completed, the analysis of the results is recently in process. While we agree with the reviewer that the pilot study data will provide additional information on the research questions, our intention to submit this manuscript is to present the main study protocol, which has different aims from the pilot study. It is our understanding that BMJ Open considers publication of study protocols that report planned or ongoing research. Although we have mentioned in the manuscript that the pilot study has preceded the main trial, we feel important to concentrate on the main study protocol. The extensive data collected from the pilot study will be presented separately in several publications after thoroughly and carefully planned analyses.

¬ Methods section;
a. It is not described in what way participants are recruited. Please define from what setting participants are to be enrolled; at the GP office, at the outpatient clinic, other?

Authors' response

We have now clarified this in the main text in the Participants subsection (pages 7 and 8).

Recruitment centres will be set up reflecting the study requirements. One recruitment centre is expected to randomise 3000 study participants, although in locations with smaller number of inhabitants, smaller than 3000 participants are acceptable. Based on the sample size calculation (see below) at least 10 centres, each recruiting 3000 study participants would be required. The study participants will be contacted through the lists that we obtain from the general practitioners (GPs), local primary care medical centres, and national medical registration databases, as appropriate, in different locations of the potential recruitment centres, keeping the population-based nature of the study as much as possible.

b. The authors do not describe in what way participants will be screened for colorectal cancer? Please define number of screening interval rounds, cut off points, number of tests, etc? Moreover, are these data also collected and published?

Authors' response

Additional text has been added to the "Interventions" section as below (page 9). Any additional rounds of the screening will be provided within locally available screening programs.

As an incentive for participation, both groups will be offered faecal occult blood testing by a laboratory-based immunochemical test (FIT) OC-Sensor (Eiken Chemical Co., Tokyo, Japan), and whenever positive (cutoff at 10 μg/g faeces from a single faecal sample), referred for colonoscopy. Any additional rounds of colorectal screening will be provided within the respective national colorectal cancer screening programs.

c. It is unclear what will be measured at the 5 year interval points. Please define what will be measured.

Authors' response

Most importantly, gastric cancer –caused mortality which is our main endpoint of the study will be

assessed at the 5-year follow-up through direct or telephone contact or alternative means of communication. A record linkage will also be made to the national Cancer and Mortality Registry database to ascertain cases of and deaths from gastric cancer. Whenever feasible, additional data on the study participants and extra biospecimen for biobanking purpose will be obtained. Additional texts have been added to the 'Interventions' section on page 10 as follows:

The groups will be followed at 5-year intervals by direct or telephone contact or alternative means of communication until the study end-points are reached. Particular attention will be given to collect detailed information on potentially H. pylori related morbidity and mortality. Whenever possible, we will invite the participants to the study centres to obtain follow-up data including demographic information, socio-economic status, physical examination as well as biological samples (plasma, serum and stool samples and gastric biopsies for microbiome testing). The new protocol will be developed to update the follow-up data collection. A record linkage will also be made to the national Cancer and Mortality Registry database to ascertain cases of and deaths from gastric cancer.

Following some minor comments:
¬ Abstract:
a. Primary and secondary objective are described in the methods section. Please place these under the introduction section or conduct a separate subheading "AIMS"

Authors' response

We thank you for the reviewer's comment. We had to comply with the Journal style for the abstract and we could not therefore create a subheading in the abstract. We have now, however, moved the objectives in the introduction section as suggested by the reviewer.

b. The incentive FIT CRC screening is not mentioned in the methods section, yet an important detail of the study design. Please mention this in the abstract method section

Authors' response

Thank you. We have now added it in the abstract Methods section.

c. The pepsinogen assay is described very unclear, please be more explicit

Authors' response

Thank you for pointing this out. The assay we are using is latex-agglutination test (Eiken Chemical Co., Tokyo, Japan). This has been clarified in the 'Intervention' section for clarity (page 8-9) as below:

The intervention group will be tested for pepsinogens (Pg) I and II by a latex-agglutination test-system (Eiken Chemical Co., Tokyo, Japan)…. Those with pepsinogen PgI/PgII≤2 and PgI≤30 ng/ml will be referred for upper endoscopy with a detailed biopsy work-up according to the updated Sydney system.

¬ Introduction:
a. In the introduction it is said gastric cancer will probably increase due to demographic changes, where will this happen and what kind of demographic changes.

Authors' response

In the Introduction, we described the global situation for gastric cancer. Despite the global decline in incidence rates over many years, the absolute burden of gastric cancer (number of cases diagnosed) has remained high as a result of population growth and aging.

We have modified the texts as below (page 5):

Most countries show declining trends in age-specific gastric cancer incidence, but the total number of cases in the world is not expected to decrease in the next decades due to demographic changes including population growth and aging.2

For your information, even if gastric cancer rates continue to decline at around the present level of approximately -2% per annum, the absolute burden is likely to remain static for the next 10-20 years because of these demographic factors. Please see Table 1 and Figure for further information. Considering that half of the gastric cancer cases in the world occur in Eastern Asia, mainly in China, substantial numbers are foreseen especially in those areas.

Table 1. Predicted gastric cancer burden 2012-2030 (Source: GLOBOCAN 2012)
Year No. gastric cancers (millions)
Demographic effect Demographic and -2.0% APC
2012 0.95 0.95
2015 1.03 0.97
2020 1.17 1.00
2025 1.34 1.03
2030 1.52 1.06

Figure Estimated number of new gastric cancer cases in 2035. Figure drawn using GLOBOCAN 2012 database; population forecasts were extracted from the United Nations, World Population prospects, the 2012 revision; numbers are computed using age-specific rates and corresponding populations for 10 age-groups.

b. The authors defined the association between H. pylori eradication and gastric cancer, and pepsinogen testing and gastric cancer, separately. It is unclear why combining H. pylori eradication with pepsinogen testing is (possibly) superior. Please elaborate more clearly if this study will be the first to study this correlation, or provide more background literature.

Authors' response

Thank you for the suggestion. Indeed, we have recognized that the principles of MAPS guidelines require better explanation in the text. We have modified the text as below (page 12 -13):

Pepsinogens are markers for atrophy of the stomach mucosa;26 decreased pepsinogen values have been demonstrated to correlate with increased risk of gastric cancer;27-29 furthermore, a combination of pepsinogen testing and H. pylori detection has been suggested to be the best available non-invasive option for gastric cancer risk stratification.13 30 However, the accuracy of pepsinogen tests to identify gastric cancer and even atrophy is imperfect.18
The current European MAPS guidelines being referred to above, are recommending surveillance of patients with precancerous lesions to enable detection of those progressing to high-risk lesions or cancer as a strategy of decreasing gastric cancer related mortality.22 However, there is still a lack of evidence from randomised control trials of combining once-per-lifetime eradication of H. pylori and

screening for high-risk conditions with blood markers such as pepsinogens for reducing gastric cancer mortality. As to our knowledge, this is the first study evaluating the yield of the above combination, i.e. mass-eradication of H. pylori and surveillance of pepsinogen-detected precancerous lesions as a strategy to reduce gastric cancer mortality.
Pepsinogen tests to identify atrophy has demonstrated a wide range of sensitivity in various studies,18 indicating that several factors may influence pepsinogen levels in different populations. The GISTAR study will allow us to investigate the role of H. pylori infection and participants' characteristics on the performance of biomarkers for identifying individuals at high risk of gastric cancer.

¬ Discussion:
a. The authors state that de GISTAR study will allow to investigate the performance of biomarkers for identifying individuals at high risk at gastric cancer. It should be noted that only positive predictive values can be calculated with this study design, and not sensitivity or specificity as might be stated in the discussion section
Authors' response

Although for the general study the reviewer's comment is absolutely true, during the piloting phase we invited a subgroup of individuals with normal biomarkers for upper endoscopy. This will enable to address not only positive predictive values, but also specificity and negative predictive value.

b. In the paragraph on trial endpoints and statistical analysis, actual numbers are not mentioned, for example: "Censoring due to mortality from other causes was taken into account using mortality rates available on the WHO mortality database for Belarus in the years 2007-2009". Please, be more explicit.

Authors' response

The sample size for this study is based on the primary variable of interest, gastric cancer mortality. Estimates of the age- and sex-specific mortality rates from gastric cancer were taken from the Globocan estimates for Belarus. Estimates of the number of deaths from gastric cancer were calculated for 5, 10, 15, and 20 years of follow-up. Censoring due to mortality from other causes was taken into account using mortality rates taken from the WHO mortality database for Belarus in the years 2007-2009 (the most recent years for which mortality data are available). In addition, a loss to follow-up of 1% per year was included in the calculations to account for migration and other reasons not related to mortality that may prevent the assessment of the primary outcome.
Table 2 summarizes the expected results from the control group, and the smallest effect of treatment that is detectable with 90% power and a significance level of 5%. The minimal detectable effect depends on time since follow-up and decreases as the number of deaths from gastric cancer accumulates. At 15 years of follow-up, 112 deaths from gastric cancer are expected in the control group, and a 35% reduction in gastric cancer mortality is detectable, corresponding to 73 cases in the intervention group.

Table 2. Expected results in the control group, minimum detectable effect of intervention at 90% power, and corresponding numbers of gastric cancer deaths in the treatment group.
Years of
follow-up N individuals under follow-up Gastric cancer deaths in control group Minimum detectable effect Gastric cancer deaths in intervention group.
0 15000 0 - 0
5 13200 29 57% 13

10 11300 67 43% 38
15 9500 112 35% 73
20 7400 158 31% 110

The changes that have been made to the text are marked in color.
In summary, I hope that we have addressed all the reviewers' comments appropriately, and are sending you the manuscript to be considered for hopefully a positive decision.

VERSION 2 – REVIEW

REVIEWER	M. Constanza Camargo National Cancer Institute, USA
REVIEW RETURNED	16-Jun-2017

GENERAL COMMENTS	The authors were responsive to my concerns and recommendations. I do not have any additional comments. I appreciate the opportunity to review this manuscript.