Peer Review File

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Reviewer A

This is an interesting prospective study in patients with P/LP CDH1 variants.

pCLE allows acquisition of optical biopsies with 1000 times magnification and has been used with high sensitivity and specificity in different diseases of the gastrointestinal tract, including esophageal squamous cell carcinoma, Barrett's esophagus, pancreatic cysts, and gastric lesions.

Comment 1: pCLE has been previously evaluated in target possible alterations, instead of screening the entire organ. Please, comment.
Reply 1: Yes, this is a true statement. We anticipated that our greatest challenge using pCLE would be to apply it to an entire at-risk organ, rather than focusing on focal abnormalities such as Barrett's esophagus. Our clinical dilemma is that there are rarely focal abnormalities present in the gastric mucosa that correspond to underlying (occult) signet ring cell carcinomas. One of our barriers to basic research is not having access to very early stage signet ring cell carcinomas in these CDH1 variant carriers, which would greatly facilitate our ability to study the pathogenesis of this rare cancer syndrome. These are the reasons why we attempted to use a technology that is often used for targeted abnormalities to instead screen an entire organ. The options for advanced endoscopic imaging are limited beyond what has already been tried, such as endoscopic ultrasound (which we don't think would be helpful), chromoendoscopy, NBI, etc.
Changes in the text: None.

Comment 2: The authors mentioned that the median procedure time was 52.5 minutes. The optimal time for pCLE examination corresponds to up 20 minutes, since this time represents the distribution of fluorescein into the capillary network and connective tissue. Did the authors inject more fluorescein after this time?
Reply 2: Thank you for pointing this out. We captured the entire procedure time, which included both pCLE examination and the collection of gastric biopsies via the Cambridge method. Although we did not specifically record time for pCLE, I can attest that it was typically about 20 minutes. For this reason, it was only on 2 occasions that we decided to re-dose the intravenous fluorescein.
Changes in the text: This has been clarified in the text, Page 7 Line 179

Comment 3: pCLE provided depth of examination of 55 to 65 μm. Do the authors believe that this could be a limitation for diagnosing signet ring cells, since they can be found more deeply?
Reply 3: This is a great question. Yes, we believe this is a potential limitation since our examination of gastrectomy explants shows that the SRC location within the lamina propria is not always as superficial as we might expect.
Changes in the text: This has been added to the discussion in the text, Page 13 Line 315-317

Comment 4: I suggest the authors to include a flowchart at the methodology to improve the study design.
Reply 4: Thank you for this excellent suggestion.
Changes in the text: We have included a flowchart, listed as “Supplemental Figure 2”.

Comment 5: Did the authors have any complication related to the fluorescein use?
Reply 5: No; there were no observed reactions related to fluorescein use.
Changes in the text: This is clarified in the text, Page 9 Line 215

Reviewer B

This study by Schuler et al aimed to evaluate cancer detection using probe-based confocal laser endomicroscopy (pCLE) during endoscopic surveillance. A total of n = 36 patients with CDH1 mutations were imaged. Contrast was generated by intravenous fluorescein. Study was controlled by non-target biopsy using Cambridge method. Signet ring carcinoma was revealed using pCLE in 16.7% (6/36) whereas 11.1% (4/36) was found with non-targeted CM biopsies.

Comment 1: How were images evaluated? Was any quantification attempted. How was signet ring carcinoma identified? Image interpretation appeared to be subjective.
Reply 1: Thank you for these questions. Yes, image interpretation was subjective as this was the first instance of pCLE for identification of occult signet ring cell carcinomas in this unique patient population. Images were evaluated in real-time by a minimum of two investigators (the principal investigator and the endoscopist). Prior to conducting the study, the confocal probe was used to evaluate explanted total gastrectomy specimens to simulate in vivo application for the purposes of the study. The study investigators appreciated the differences in uniformity of gastric pits and intervening stroma of normal mucosa and aimed to identify areas that did not appear normal. Thus, the criteria could simply be described as identifying the abnormal; however, no objective criteria were derived and no quantification was attempted.
Changes in the text: Addressed in Discussion; Page 13, Line 316-319

Comment 2: The working distance of the confocal endomicroscope was not stated. Were the images collected at an imaging depth sufficient to identify signet ring carcinoma?
Reply 2: This is an excellent question, thank you. The manufacturer lists the visual depth of the confocal probe to be 55-65 microns, however we do not know the working depth in vivo when placed on the surface of the gastric epithelium especially as it relates to the depth sufficient to visualize occult signet ring cell carcinomas. This is clearly a limitation of the study and we will add some clarification to the text.
Changes in the text: Added to the discussion section, Page 13 Line 315-316

Comment 3: The authors conclude that pCLE has low sensitivity for gastric cancer detection. What is the role for this imaging technology in surveillance. Why did the authors perform the study?

Reply 3: Thank you for these questions. Yes, based on our small study, we are obliged to conclude that pCLE is not very sensitive as it was used and interpreted in these patients. However, the role of pCLE in early cancer surveillance in hereditary diffuse gastric cancer may not be fully determined after this lone study. We performed this study in part to determine if the technology could have a role in surveillance. We suspect that with improvements in image interpretation – such as with artificial intelligence – that it could be. The other reason we performed the study was to determine if pCLE could help provide a reliable method for identifying signet ring cells in vivo during the potential conduct of a chemoprevention study, wherein an experimental drug would be conjugated with a fluorophore for tracking. Thus, while potentially not clinically useful (for patients or their doctors), the technology could prove useful for translational research.
Changes in the text: None.