Peer Review File

Article Information: https://dx.doi.org/10.21037/jgo-21-505

1. While it makes sense to exclude patients with prior neoadjuvant chemotherapy to capture de novo PD-L1 status, for the sole purpose of comparing staining characteristics between two antibodies, why not also include patients with prior chemotherapy to increase statistical power to detect any differences.

We thank the reviewer for fruitful suggestions. The reason why the patients who received prior neoadjuvant chemotherapy was that it was reported the PD-L1 status may change after chemotherapy. We would like to assess the survival according to the PD-L1 status. If the PD-L1 status changes after the chemotherapy, it may affect the results of survival and the patients were excluded from this study.

2. Ideally as a predictive biomarker there should be linkage to response to immune checkpoint inhibitors. While this was a resected earlier stage disease population, any clinical annotation available on patients who may have received anti-PD-1 therapy and correlate with survival in the few cases that had discrepancy between assays?

The anti-PD-1 antibody was approved in 2017 in Japan. The study included the patients who received gastrectomy between 2009-2010. In almost all cases of cancer recurrence, the patients died without receiving anti-PD-1 antibody. There is a gap between the time when the anti-PD-1 antibody was approved and the time of this cohort. Therefore, we do not have any data on the patients who received anti-PD-1 antibody in this cohort.

Reviewer B

1. Given the intention to compare immunostainings, a decent and meticulous description of the methods is of paramount importance. This includes a detailed description of dilutions used, staining kits and controls (positive and negative).

We thank the reviewer for fruitful suggestions. Positive-control and negative-control are used for non-small cell lung cancer cases (two cases with known PD-L1 status) from our own institution. According to this comment, we attached the excel file as a supplementary file about details of IHC antibodies used in this study.

2. How evaluation was done, is not described. The first author seems to be a clinician. However, the study is primarily a histopathological analysis and first of all qualification of the contributors needs to be illustrated. Were board certified surgical pathologists involved? How was inter- and intraobserver rating considered and assessed?

According to this comment, we added the sentences “ES and YY evaluated the immunostaining as board certified pathologists” (page 8, lines 146–147).

3. Statistics lack correction for multiple testing.

Thank you for your kind comment. According to this comment, we added the sentences "Although there may be a lack of correction for multiple comparisons in statistics, it still remains difficult to select a proper method suitable for the various experimental properties." (page 13, lines 271–273).

4. Overall, the number of patients is low and compromises the validity of the data.

Thank you for your kind comment. We pointed out this limitation in the sentences "This was a retrospective study with a small number of cases from a single institution" (pages 13, lines 270–271).

5. The data of E1L3N should be added to main the results and not hidden in the supplemental data.

Thank you for your kind comment. The main purpose of this study is to evaluate the programmed death ligand-1 staining patterns of gastric adenocarcinoma evaluated by the 22C3 and 28–8 pharmDx assays. Therefore, we think the data of E1L3 is not necessary in the main manuscript to reveal the discrepancy of staining patterns between 22C3 and 28–8 assays. In addition, the supplementary data is clearly shown on the web page and we are not trying to hide anything.

6. Figure 1 is of low quality. This could be related to the conversion into a pdf-file. However, staining quality cannot be assessed in the document provided. The figure legend fails to describe magnifications.

According to this comment, we added the sentences "The photo shows magnification 200x." (page 21, lines 425–426).