Peer Review File

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Reviewer A

Gits et al determined the frequency of intact SMAD4 and its association with patterns of recurrence in patients with upfront resected pancreas cancer. Of 127 patients 10 (8%) patients had intact SMAD4 expression. Grade was the only clinicopathologic parameter statistically associated with SMAD4 expression. On multivariable analysis, SMAD4 expression and adjuvant chemoradiotherapy were associated with higher and lower locoregional recurrence, respectively.

This is a well written paper on a clinically relevant topic. The findings are not novel. Currently, SMAD4 status is one example of how underlying genetic status can correlate with biological behavior in PDAC: PDAC not expressing SMAD4 is associated with a high metastatic burden, while tumors with intact SMAD4 tend to remain localized.

Comment 1: How was locoregional recurrence defined? (recurrence in the remnant pancreas or in the surgical bed, such as soft tissue along the celiac or superior mesenteric artery, aorta or around the pancreaticojejunostomy site, cfr Groot et al, Ann Surg 2018, Patterns, timing, and predictors of recurrence following pancreatectomy for pancreatic ductal adenocarcinoma).
Reply 1: This comment addresses some ambiguity in the methods section in definitions of recurrence and recording of recurrence events. Definitions of both locoregional and distant recurrence were provided in the text, and a statement was added to clarify how recurrence events were recorded (page 9, lines 175-179).

Comment 2: 20 patients had locoregional recurrence, 80 patients had distant recurrence, 12 patients had both. In Table 2 the total number of patients with recurrence is 88 "Any recurrence"? However, 20+80+12 =102. Something that I do not understand? Reccurence is either locoregional, distant or both locoeregional and distant.
Reply 2: As originally written in the text, there is room for misunderstanding. In this study, associations of clinicopathologic features with the type of recurrence (locoregional or distant) were made independently. Thus, locoregional and distant recurrences were tracked independently. Patients could be characterized as having one or both types of recurrence, whether identified concurrently or sequentially. Reply 1 partially addresses this issue. To clarify these points and the intended analysis further, text has been added to the results section (page 10, lines 207-210). Also, Table 2 has been rearranged to demonstrate "Any recurrence" encompasses the subsequent types (page 28 line 526).

Comment 3: Table 1. Tumor site. (n=1) is missing after 10 % in Overlap/Not specified.
Reply 3: This error has been corrected in Table 1 (page 26, line 523).

Comment 4: All patients received adjuvant gemcitabine. Why is not “Adjuvant chemotherapy (gemicitabine) Yes/No added in Table 1? In most single centre studies 20-30 % of patients do not initiate adjuvant therapy due to postoperative complications, poor performance status or early disease progression. Is the initation rate of adjuvant therapy after pancreatectomy for pancreatic cancer 100 % at the study hospital? Probably the title of the paper should be changed to: "Intact SMAD-4 is a Predictor of Increased Locoregional Recurrence in Patients with Upfront Resected Pancreas Cancer Receiving Adjuvant Therapy").

Reply 4: While not all patients at the study hospital receive adjuvant gemcitabine, the subset of patients who received adjuvant gemcitabine were chosen for the study population to isolate better the potential association of SMAD4 status and pattern of recurrence. Differences in treatment paradigm from the historical standard of care, such as the omission of adjuvant chemotherapy, could obscure the study question. The discussion has been updated to express the rationale for the selected subset (pages 11-12, line 238-241). Additionally, the title (page 2, line 27) and abstract (page 4, lines 75, 79) have been updated to state that all patients received adjuvant therapy.

Comment 5: Reference 57 (Versteijne et al) did not show any survival benefit of upfront versus neoadjuvant therapy except in a subgroup analysis of borderline resectable cases. This should be corrected in the Discussion.

Reply 5: The manuscript as previously written is not clear in conveying some nuances from the PREOPANC trial and the particular subgroups of interest. The PREOPANC trial performed multiple subgroup analyses, including comparison of borderline resectable cases, as mentioned in Comment 5. Another subgroup analysis compared of patients with tumor resection who started adjuvant treatment and the immediate surgery group. From the text of Versteijne et al., “The predefined subgroup of patients with tumor resection who started adjuvant treatment showed a significantly improved median OS of 35.2 months (95% CI, 26.2 months to not available) in the preoperative chemoradiotherapy group and 19.8 months (95% CI, 16.8 to 32.2 months) in the immediate surgery group (HR, 0.58; 95% CI, 0.35 to 0.95; P = .029)”. Text has been added to the discussion to clarify the specific subgroup and comparison of interest (page 12, lines 260, 262).

The Discussion is interesting to read. The authors acknowledge the small study sample and that the findings are not novel. However, they discuss the clinical implications of their findings and how we may bring this research topic forward.

Reviewer B

In the presented manuscript, the authors report a rather surprising finding; that intact SMAD4 associates with locoregional recurrence in PDAC, and tumor grade.

I am surprised by the 8% intact SMAD4 in the cohort (the authors mention previously reported frequencies in the Introduction, and elaborate on it in the Discussion). This results in very

asymmetrical groups in the comparison, with one being likely underpowered.

This lack of power might hide the association of SMAD4 with important clinical variables that could explain why the authors come to such an unexpected finding. See for instance N1 stage in Table 1, and CA19-9.

Comment 1: Given the above considerations, a validation cohort is required. Again, the authors mention this in their Discussion but it seems to this reviewer that it wouldn't be very hard to find a TMA elsewhere to validate (part of) the results?
Reply 1: This comment addresses a limitation within the study, namely the unexpectedly low frequency of intact SMAD4. As acknowledged, validation of these findings with a separate clinical cohort is crucial. Obtaining and analyzing such a cohort, however, is not trivial and is a focus of active work by the investigators. These results are unable to be completed within the timeline of a manuscript revision. While lacking a true validation cohort, the findings from this study fit within the context previous literature. These points are now expanded in the discussion to promote a bridge to future study (page 14, lines 298-301).