Peer Review File

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Reviewer A

Comment 1:

In this single-center retrospective study, the authors evaluated the efficacy of 5-FU/LV + nal-IRI in a daily practice, especially among the patients who underwent 5-FU/LV + nal-IRI as third-line chemotherapy. The authors concluded that the use of 5-FU/nal-IRI seemed to be beneficial in third-line therapy, despite a pre-exposure to non-liposomal irinotecan. However, the data the authors showed in this manuscript is not enough to show the benefit of 5-FU/nal-IRI in the patients who had already undergone irinotecan, mainly due to the heterogeneity of study patients.

They included the patients whose M stage was 0 or 1, those who had undergone curative surgery, and those who underwent concomitant local therapy. These patient background affected prognosis and treatment outcomes.

Therefore, it is difficult for readers to agree with the conclusion in the abstract based on the data in this manuscript.

According to the reason mentioned above, I concluded "Reject" is the suitable decision for this manuscript.

Reply 1: We agree with the reviewer, that one of the main limitations of the study is the small number of patients. However, in our opinion, even a case report on the use of 5-FU/NalIRI in the context of clinical practice as a third line therapy after FOLFIRINOX and gemcitabine-base therapy is valuable if it contributes to the understanding of how to treat patients with advanced pancreatic cancer.

With respect to tumor stage heterogeneity, all patients included in the study received all systemic therapies in the setting of advanced stage (at first diagnosis or at recurrence diagnosis). Therefore, the prognosis of all patients with advanced pancreatic cancer whether first diagnosed or as a recurrent diagnosis, must be considered similar and very poor in all cases, regardless of initial curative surgery. Local ablative or endoscopic therapies were performed to palliate local symptoms such as pain, duodenal stenosis or biliary congestion. These therapies are well-known not to influence the prognosis of the patients with advanced pancreatic cancer and were balanced between the two groups of patients.

However, being aware of the fact, that some baseline parameter might affect the prognosis and treatment outcomes, we have included the parameters of initial surgery and local therapies in the uni- and multivariate analysis to rule out a possible influence on the prognosis and outcome of the therapy with 5-FU/NalIRI.

Changes in the text: We have added more explanation about the administration of all systemic therapies in the setting of advanced stage of disease in the “Data collection and study design” section. Moreover, we further adjusted the limitations of the study (see Page 16, line 5). Finally, we expanded our uni- and multivariate analysis and added the undergone

surgery and concomitant local therapies (see page 10, line 14-15; see table 3).

Reviewer B

Comment 1:

1. The sample size is too small to make any conclusion. And there is no new information.

Reply 1: We agree with the reviewer, that one of the main limitations of the study is the small number of patients. However, in our opinion, even a case report on the use of 5-FU/nal-IRI in the context of clinical practice as a third line therapy after FOLFIRINOX and gemcitabine-base therapy is valuable if it contributes to the big picture and larger scientific understanding of how to treat patients with advanced pancreatic cancer.

Therefore, we tested and evaluated current points of interest concerning therapy with 5-FU/nal-IRI in pancreatic cancer.

Changes in the text: we further adjusted the limitations of the study (see Page 16, line 5-10).

Comment 2:

2. Concomitant local therapy such as HIFU were added during nal-IRI/5-FU? Why did you add local treatment? Did it affect response rate or survival outcome? Did you exclusion measurable lesion treated with local therapy?

Reply 2: Local ablative or endoscopic therapies were performed to palliate local symptoms such as pain, duodenal stenosis or biliary congestion. These therapies are well-known not to influence the prognosis of the patients with advanced pancreatic cancer and were balanced between the two groups of patients.

However, being aware of the fact, that some baseline parameter might affect the prognosis and treatment outcomes, the reviewer is right and we have included the parameters of local therapies in the uni- and multivariate analysis to rule out a possible influence on the prognosis and outcome of the therapy with 5-FU/nal-IRI.

Changes in the text: We included the local palliative therapies within our univariate analysis, and it showed no statistically significant influence on overall survival (see table 3).

Comment 3:

3. In result session, the authors described that they reduced starting dose of nal-IRI/5-FU.

It is unclear if they reduced the entire regimen or nal-IR, the % of reduction, and the reason of dose reduction. The reason of dose modification and the dose intensity of each drug should be described.

Reply 3: 24.1% of patients receiving 5-FU/nal-IRI presented a reduced performance status (ECOG 2-3) before starting the 2nd line/3rd line therapy with 5-FU/nal-IRI. Usually, in these cases and in cases of persistent side effects of previous therapies, such as diarrhea and/or bone marrow suppression, therapy with 5-FU/nal-IRI was started with reduced dose (50% dose) and was augmented to 100% in the second course of therapy, if therapy did not worsened the performance status or side effects. In 37.9% of patients dose reduction was necessary at starting therapy. 55.2% of patients have dose modifications in further courses of therapy, of which 20.7% of patients had reduced the entire regimen of 5-FU/nal-IRI by 50% dose.

Changes in the text: Further informations about the dose modifications of 5FU/nal-IRI were

included in the result section (s. page 11) as required by the reviewer.

Comment 4:

4. It is important to analyze the clinical outcome in patients treated with previous FOLFIRINOX.

It's more helpful that the authors described the data with the information of previous FOLFIRINOX treatment, such as the number of cycle of FOLFIRINOX, response, duration of response, time interval between FOLFIRINOX and nal-IRI/5-FU. And also the response of nal-IRI/5-FU should be analyzed according to previous response to FOLFIRINOX.

Reply 4: The Reviewer is right, the therapy information about FOLFIRINOX-treatment should be displayed. Therefore, we added data concerning the FOLFIRINOX pretreatment. The analysis of nal-IRI response (Disease control rate, DCR) depending on FOLFIRINOX response was analyzed with a binomial logistic regression. The binomial logistic regression model was not statistically significant, $\chi^2(2) = 3.627$, $p = 0.163$ and had a low amount of explained variance (Nagelkerkes $R^2 = 0.317$). Due to the weak data, we did not present these results to make it easier to understand the already presented data.

Changes in the text: We added data to table 1 (see table 1).

Comment 5:

5. In discussion session, authors suggested that nal-IRI/5-FU showed similar anti-cancer benefit even in patients exposure to FOLFIRINOX. However, there was no data to support this conclusion. What data or evidence can you suggest?

Reply 5: Since the 19 patients receiving 5-FU/nal-IRI as 3rd line therapy achieved a mOS of 9.33 months (95 %CI: 3.39, 15.28), similar to the survival achieved by patients receiving 5-FU/nal-IRI as 2nd line therapy, a benefit due to 5-FU/nal-IRI in this setting can be suggested in our cohort of patients. However, the reviewer is right and the study is underpowered to compare the both groups of patients. Therefore, lack of significance between both groups of patients could be only related to the low number of patients. Therefore, we cannot perform any statement about the benefit of this therapy, and our suggestion that this therapy may be beneficial should be taken with caution.

Changes in the text: The relevant limitation of the study was added in the discussion, accordingly (s. page 16).

Comment 6:

6. The authors described the study data in discussion. Those paragraph has to be moved the result session. (for example, toxicity)

Reply 6: The reviewer is right; we describe data concerning adverse events in the discussion session that are only referred to the results section by pointing to table 4. We presented our data again in the discussion section to make it for the reader easier to compare the results with the NAPOLI1-trial results.

Changes in the text: We added the data within the results section (see page 10, line 20).

Reviewer C

I commend the authors for asking an important clinical question and seeking to provide data where there has previously been relatively few. Overall, the study is underpowered, but can, I think, still contribute to the larger scientific understanding of how best to treat patients with such a dire condition as advanced pancreatic cancer. I hope you will consider the following questions and suggestions.

Comment 1:
-When describing the study design and demographics of included patients, consider acknowledging that all previous treatments were administered in the setting of advanced disease. This is mentioned in the discussion, but I imagine other readers will come to this question, as I did, at this place in the manuscript.
Reply 1: The reviewer is right, we shall make it more clear that previous therapies were administered in the advanced disease setting.
Changes in the text: We added the required information in the "Data collection and study design"-section (see page 5, line 20 and line 25-26).

Comment 2:
-Therapy response assessment via imaging was described as occurring every 8-12 weeks. Acknowledging the retrospective nature of this work, I wonder if there is a strong shared practice (e.g., efforts were made to schedule imaging every 8 weeks, but an interval of up to 12 weeks was acceptable) or not. Although PFS was not the primary outcome, the median PFS estimate was short enough, and with a small enough difference between cohorts, that timing of assessment could have affected the results.
Reply 2: There was intention to schedule imaging every 8 weeks in order to determine, whether the last line therapy reached any response. However, this was not possible in all cases.
Changes in the text: This information was included in the methods section (s. page 6).

Comment 3:
-On p6, line 16, the FOLFIRINOX regimen is described as being administered every 15 days, is that accurate?
Reply 3: The therapy with FOLFIRINOX will be repeated on day 15 of the two weeks cycle.
Changes in the text: We changed the wording to weeks to make it more clear (see page 6, line 18).

Comment 4:
-Was gemcitabine (either with or without nab-paclitaxel) always administered on the same schedule? Days 1, 8, and 15 q28d?
Reply 4: The standard therapy rhythm was a cycle of 4 weeks with therapy administration in days 1, 8 and 15.
Changes in the text: We added the exact therapy schedule in the methods section (see page 6, line 22)

Comment 5:
-When describing nal-IRI dose, consider specifying that 80 mg/m2 is equivalent to 70 mg/m2

irinotecan base. In the US prescribing information, nal-IRI is described based on the irinotecan base component, so this may help eliminate confusion.
Reply 5: We added the required information in the text for more clarification.
Changes in the text: The information that 80 mg/m2 nal-IRI is equivalent to 70 mg/m2 irinotecan base was added in the methods section (s. page 7)

Comment 6:
-On p8, lines 14-15 it is stated that 94.7% of subjects in the 3L cohort received gemcitabine-based therapy in 2L. Should this percentage be 84.2% consistent with the previous sentence and with Table 1?
Reply 6: The reviewer is completely right, the 94.7% resulted out of the accidentally summation of FOLFIRINOX and gemcitabine/nab-paclitaxel. The true percentage is 84.2%.
Changes in the text: We corrected the value (see page 8, line 20).

Comment 7:
-On p9, Lines 11-16 there is a description of survival outcomes among patients in the 3L cohort based upon previous progression on irinotecan or not. It is curious to me that the median survival of both subgroups is substantially below that of the cohort overall (5.6 mo and 7.5 mo vs. 9.33 mo). This does not seem possible unless everything hinged on the one patient excluded from the analysis. If these numbers are correct, it would help to know the survival of that patient in question and to see the survival curves for censoring.
Reply 7: The excluded patient had an overall survival of 34.1 months and was still alive by the end of the observation period. By artificially adding the patient to the "without progression" group the OS is 10.5 months and 5.6 months in the "with progression" group resulting in the total OS of 9.3 months. Vice versa the results were as following: "without progression" 7.5 months and "with progression" 9.33 months.

Original results:

Überlebenstabelle[a]

GrundAbbruchFOLFIRINOX		Zeit	Status	Kumulierter Anteil Überlebender zum Zeitpunkt: Schätzer	Kumulierter Anteil Überlebender zum Zeitpunkt: Standardfehler	Anzahl der kumulativen Ereignisse	Anzahl der verbliebenen Fälle
Toxizität	1	2,333	ja	,750	,217	1	3
	2	7,533	ja	,500	,250	2	2
	3	10,533	ja	,250	,217	3	1
	4	10,533	nein	.	.	3	0
Progress	1	1,100	ja	,929	,069	1	13
	2	1,400	ja	,857	,094	2	12
	3	2,067	ja	,786	,110	3	11
	4	2,200	ja	,714	,121	4	10
	5	2,367	ja	,643	,128	5	9
	6	3,267	ja	,571	,132	6	8
	7	5,567	ja	,500	,134	7	7
	8	5,600	nein	.	.	7	6
	9	5,867	nein	.	.	7	5
	10	8,233	nein	.	.	7	4
	11	9,333	ja	,375	,148	8	3
	12	10,400	nein	.	.	8	2
	13	14,367	ja	,188	,152	9	1
	14	20,267	ja	,000	,000	10	0

a. Gruppe = NAPOLI 3rd Line

Mittelwerte und Mediane für die Überlebenszeit[a]

GrundAbbruchFOLFIRINOX	Mittelwert[b]				Median			
			95%-Konfidenzintervall				95%-Konfidenzintervall	
	Schätzer	Standardfehler	Untere Grenze	Obere Grenze	Schätzer	Standardfehler	Untere Grenze	Obere Grenze
Toxizität	7,733	2,051	3,713	11,754	7,533	4,100	,000	15,569
Progress	8,944	2,194	4,644	13,244	5,567	4,127	,000	13,656
Gesamt	8,735	1,781	5,243	12,227	7,533	3,037	1,580	13,487

Artificially adding the cancelled patient to “without progression”(referred as “Toxizität”):

Überlebenstabelle[a]

GrundAbbruchFOLFIRINOX		Zeit	Status	Kumulierter Anteil Überlebender zum Zeitpunkt: Schätzer	Kumulierter Anteil Überlebender zum Zeitpunkt: Standardfehler	Anzahl der kumulativen Ereignisse	Anzahl der verbliebenen Fälle
Toxizität	1	2,333	ja	,800	,179	1	4
	2	6,833	nein	.	.	1	3
	3	7,533	ja	,533	,248	2	2
	4	10,533	ja	,267	,226	3	1
	5	10,533	nein	.	.	3	0
Progress	1	1,100	ja	,929	,069	1	13
	2	1,400	ja	,857	,094	2	12
	3	2,067	ja	,786	,110	3	11
	4	2,200	ja	,714	,121	4	10
	5	2,367	ja	,643	,128	5	9
	6	3,267	ja	,571	,132	6	8
	7	5,567	ja	,500	,134	7	7
	8	5,600	nein	.	.	7	6
	9	5,867	nein	.	.	7	5
	10	8,233	nein	.	.	7	4
	11	9,333	ja	,375	,148	8	3
	12	10,400	nein	.	.	8	2
	13	14,367	ja	,188	,152	9	1
	14	20,267	ja	,000	,000	10	0

a. Gruppe = NAPOLI 3rd Line

Mittelwerte und Mediane für die Überlebenszeit[a]

GrundAbbruchFOLFIRINOX	Mittelwert[b]				Median			
			95%-Konfidenzintervall				95%-Konfidenzintervall	
	Schätzer	Standardfehler	Untere Grenze	Obere Grenze	Schätzer	Standardfehler	Untere Grenze	Obere Grenze
Toxizität	8,093	1,769	4,627	11,560	10,533	3,471	3,730	17,336
Progress	8,944	2,194	4,644	13,244	5,567	4,127	,000	13,656
Gesamt	8,996	1,777	5,512	12,480	9,333	3,033	3,388	15,279

Artificially adding the cancelled patient to “with progression”(referred as “Progress”):

GrundAbbruchFOLFIRINOX		Zeit	Status	Kumulierter Anteil Überlebender zum Zeitpunkt Schätzer	Standardfehler	Anzahl der kumulativen Ereignisse	Anzahl der verbliebenen Fälle
Toxizität	1	2,333	ja	,750	,217	1	3
	2	7,533	ja	,500	,250	2	2
	3	10,533	ja	,250	,217	3	1
	4	10,533	nein	.	.	3	0
Progress	1	1,100	ja	,933	,064	1	14
	2	1,400	ja	,867	,088	2	13
	3	2,067	ja	,800	,103	3	12
	4	2,200	ja	,733	,114	4	11
	5	2,367	ja	,667	,122	5	10
	6	3,267	ja	,600	,126	6	9
	7	5,567	ja	,533	,129	7	8
	8	5,600	nein	.	.	7	7
	9	5,867	nein	.	.	7	6
	10	6,833	nein	.	.	7	5
	11	8,233	nein	.	.	7	4
	12	9,333	ja	,400	,151	8	3
	13	10,400	nein	.	.	8	2
	14	14,367	ja	,200	,160	9	1
	15	20,267	ja	,000	,000	10	0

a. Gruppe = NAPOLI 3rd Line

Mittelwerte und Mediane für die Überlebenszeit[a]

GrundAbbruchFOLFIRINOX	Mittelwert[b] Schätzer	Standardfehler	95%-Konfidenzintervall Untere Grenze	Obere Grenze	Median Schätzer	Standardfehler	95%-Konfidenzintervall Untere Grenze	Obere Grenze
Toxizität	7,733	2,051	3,713	11,754	7,533	4,100	,000	15,569
Progress	9,369	2,187	5,082	13,655	9,333	4,567	,382	18,284
Gesamt	8,996	1,777	5,512	12,480	9,333	3,033	3,388	15,279

a. Gruppe = NAPOLI 3rd Line

Changes in the text: none

Comment 8:

-When describing the survival analysis from the beginning of advanced disease therapy, there is no mention of immortal time bias. Please consider acknowledging this.

Reply 8: The reviewer is right, the immortal time bias should be acknowledged in the context of analysis of survival from the beginning of advanced disease.

Changes in the text: We added some information in the discussion section (see page 14, line 24)

Comment 9:

-In the Discussion, please consider expanding upon the notion that prior exposure to irinotecan is not the same as resistance to irinotecan. This is touched upon in the manuscript, and was even evaluated within your data set, but I think the point could be stated more clearly. In my reading there were points where this line was blurred (i.e., p13, lines 5-7)

Reply 9: The reviewer is right, it is important to clearly differentiate between irinotecan exposure and irinotecan resistance.

Changes in the text: We added a subclause on page 13, line 5 and rearranged the informations in the following section to make the point more clear (see page 13, line 6-11).

Comment 10:

-On p11, line 9-10, it is unclear in the sentence describing 1L regimens whether a specific sequence is being recommended, or if the sentence is simply listing the two preferred options for 1L treatment.
Reply 10: In this sentence we presented the two possible options and did not intend to suggest a sequence.
Changes in the text: We changed the word "and" to "or" (see page 11, line 17).

Comment 11:
-A published study you may have missed is Tossey et al in Medical Oncology 2019 (PMID: 31494781). Overall, that real-world description of nal-IRI fits very well with your findings and the other literature you cite, but it would change slightly the range of PFS in published literature (4.1 months)
Reply 11: The mentioned study is definitely an enrichment for our manuscript.
Changes in the text: We added the citation (see page 12, line 17, citation number 25) and changed the range of PFS in the published literature (see page 12, line 18)

Comment 12:
-When describing the comparison between 2L and 3L cohorts in this study, there is no mention of the fact that none of the patients in the 2L cohort received 3L+ therapy, and that none were ever treated with platinum and few (20%) were treated with fluoropyrimidine. Is this typical of your practice site or were these patients all deemed unfit for further therapy? Although not found to be prognostic in this limited data set, it is clear that patients in the 2L cohort were older and received less treatment overall (i.e., no 3L therapy and more common nal-IRI reductions) so it is informative that these patients still had numerically better survival outcomes compared to the 3L cohort. Could some, perhaps, have benefitted from additional treatment after nal-IRI/5FU?
Reply 12: The patients (n=10) of the 2L group did not wish any further therapy or it was contraindicated due to bad performance status (ECOG ≥ 3) and/or insufficient organ function. We agree with the reviewer, and some patients can benefit from a third line therapy after 5-FU/nal-IRI. In our practice, we therefore perform therapies with the OFF or Gem/Ox regimes as last line therapy, if the patients can be qualified for this therapy.
Upfront, a determination of BRCA1/2 mutation status will be performed in all patients. In case of positive finding, a platinum therapy is offered to the patients in any case, preferably in the first line therapy. None of our patients in the 2L cohort was BRCA-mutated.
Finally, all patients of our cohort received fluoropyrimidine at least during the NAPOLI-1 protocol and not only 20% (this 20% patients received additional capecitabine during a past adjuvant therapy with gem/cap). Therefore, a re-induction with 5-FU or capecitabine after NAPOLI-1 is usually not offered.

Changes in the text: None

Comment 13:
-When describing published comparisons of survival based upon prior irinotecan exposure (p12, line 25 through p13, line 1) the order of the numerical results does not match the order

of the cohorts as ≤described in the sentence text.
Reply 13: We double-checked the described results and the results are assigned as follows:
Barzi: 5.6 vs. 4.1 (page 4 of the publication PDF)
Yoo: 10.2 vs. 4.4 (page 5 of the publication PDF)
Macarulla Mercadé: 6.7 vs. 4.6 (page 8 of the publication PDF).
But the reviewer is correct, the order of the citation at the end of the sentence does not match the results, but this is due to earlier citation of the sources.
Changes in the text: None

Comment 14:
-This paragraph is also an opportunity to provide more nuance and discussion of the distinction between prior exposure and prior disease progression
Reply 14: The reviewer is right, this issue needs to be pointed out in particular.
Changes in the text: We summed the paragraph up to make this point more clear (see page 13, line 18-20).

Comment 15:
-On p14 there is discussion of survival from the time of diagnosis of advanced disease. Was time from diagnosis of advanced disease until start of nal-IRI different between 2L and 3L groups? And, assuming so, was it prognostic for survival in this data set?
Reply 15: The time from beginning of advanced disease therapy was statistically significant different between both groups but did not show influence on OS after starting 5-FU/nal-IRI in univariate analysis.
Changes in the text: We added the above mentioned data to table 1 and table 3.

Comment 16:
-Furthermore, this comparison within this dataset is not balanced because none of the patients in the 2L cohort received 3L+ therapy. Hatashima et al (PMID: 35579260) noted that survival was similar between cohorts of patients who received all of the "available" classes of chemotherapy (i.e., fluropyrimidine, irinotecan (conventional or liposomal), oxaliplatin, gemcitabine, and nab-paclitaxel) regardless of the sequence in which these agents were administered.
Reply 16: As mentioned in reply 12, one patient in the 2L cohort received a third line therapy with gemcitabine/erlotinib. However, the reviewer is right, the groups are not balanced in this parameter. Therefore, our dataset is not suitable for answering the question of optimal therapy sequence or amount of therapy lines in terms of overall survival. We only picked this issue out as it is a relevant clinical question and correlated published data to our results in order to support the previous findings.
Changes in the text: In the mentioned paragraph (page 14, line 23-24) the correlating sequence of therapy by Glassman et al. to our therapy groups was mixed up, we fixed the mistake.

Comment 17:
-When describing limitations of this work, and the small sample size, consider including a

reverse power calculation since this study was underpowered and all statistical comparisons should be understood within that context.

Reply 17: The author is right, the study is underpowered. This work (http://dx.doi.org/10.1136/gpsych-2019-100069) analyzed the performance of post-hoc analysis and found it not indicating true power. We prefer to list the small sample size as one of the major limitations of this study. For completeness, the power analysis of the log rank test for overall survival showed a very low power of 0.247.

Changes in the text: None