Author’s response to reviews

Title: Nal-IRI with 5-fluorouracil (5-FU) and leucovorin or gemcitabine plus cisplatin in advanced biliary tract cancer - The NIFE trial (AIO-YMO HEP-0315) an open label, non-comparative, randomized, multicenter phase II study

Authors:

Lukas Perkhofer (lukas.perkhofer@uniklinik-ulm.de)
Andreas Berger (andreas.berger@vivantes.de)
Alica Beutel (alica.beutel@uniklinik-ulm.de)
Eike Gallmeier (eike.gallmeier@med.uni-marburg.de)
Stefan Angermeier (stefan.angermeier@kliniken-lb.de)
Ludwig Fischer von Weikersthal (weikersthal.ludwig@gesundheitszentrum.klinikum-amberg.de)
Thorsten Goetze (Goetze.Thorsten@khnw.de)
Rainer Muche (rainer.muche@uni-ulm.de)
Thomas Seufferlein (thomas.seufferlein@uniklinik-ulm.de)
Thomas Ettrich (thomas.ettrich@uniklinik-ulm.de)

Version: 1 Date: 01 Aug 2019

Author’s response to reviews:

Dear Reviewers,

We are happy that our manuscript entitled "Nal-IRI with 5-fluorouracil (5-FU) and leucovorin or gemcitabine plus cisplatin in advanced biliary tract cancer - The NIFE trial (AIO-YMO HEP-0315) an open label, noncomparative, randomized, multicenter phase II study", nearly fulfills criteria for publication in BMC Cancer.

Therefore we really appreciate to address the questions raised by the reviewer and believe that they improve the quality of the paper. Attached you can find the changes made commented in a point by point form.
Editor Comments:
1. Please clarify in the title page who the corresponding author is, there appears to be a symbol next to one of the author's name, but no key as to what that symbol means.
Answer: The corresponding author (Lukas Perkhofer) is now clearly highlighted on the title page.

2. Please provide figure titles/legends under a separate heading of 'Figure Legends' after the References. If Figure titles/legends are within the main text of the manuscript, please move them.
Answer: A separate heading is added following the references, with the title and legend of figure 1. Figure files should contain only the image/graphic, as well as any associated keys/annotations. If titles/legends are present within the figure files, please remove them.
Answer: A separate file was created and uploaded.

3. Table 1 is not really in a table format, could we please request that this information simply be incorporated into the manuscript or the table to be reformatted to appear as a table.
Answer: The information of previous Table 1 is now incorporated into the text (page 6, line 1-32).

4. As "Trial status" and "Related Articles" are not standard sections in the Declarations, we thank you for adding this information, and would ask that you move the information in these two sections to the Acknowledgments section.
Answer: The previous information of trial status and related articles are moved to the acknowledgment section.

Reviewer reports:

Reviewer 1:

1) The background discussion should describe in more detail the prior irinotecan based protocols in BTC. What was the PFS at 4 months on those trials and with the current standard of care? A table would be useful to summarize this data.
Answer: We absolutely agree with the reviewer that the data basis should be strengthened. However, in preparation of the current manuscript and the study protocol we did an expanded review of the literature. All relevant MEDLINE listed trials are actually included into the manuscript. This did not change after performing anew a literature research in preparation for this revision. We added the reference of another trial in progress using FOLFIRINOX as second line treatment in advanced BTC presented at ASCO 2019 being available as abstracts but not fully published, that helps to underline the use of irinotecan based regimen (page 10, line 21).

2) What is the clinical significance of PFS at 4 months? Why was this chosen as the primary endpoint. I suspect it was chosen for statistical / sample size reasons, but it seems very arbitrary.
Answer: We agree with the reviewer as PFS 4-months is not a common trial end-point. For this phase 2 trial
we choose the 4-months PFS rate as an endpoint for several reasons: i) the largest phase II trial in the field
the BINGO trial had the same endpoint, allowing better comparability, ii) OS has proven to be not a
sufficient endpoint in BTC trials (Eckel et al., Br J Cancer 2007) and iii) the time point was chosen to allow
earliest possible comprehensive interims analysis for safety and efficacy reasons.

3) A rational for the use of Nap-IRI in BTC should be better delineated. The fact that it worked in advanced pancreas cancer is not sufficient.
Answer: This trial has to justify the rationale for the use of Nal-IRI in BTC as far as there is no pre-clinical or
clinical published data by now. This is equivalent to the trials running on FOLFIRINOX in BTC.

4) There is quite extensive literature / current work related to targeted therapy in BTC. Up to 30% of these
cancers have a targetable mutation / alteration (FGFR, HER2, BRAF, etc). This should be reviewed /
addressed in the manuscript and in the discussion in relation to planned correlative studies.
Answer: Recent sequencing efforts definitely improved biological knowledge on BTC. However, the
obtained results clearly emphasized the discrepancy of intrahepatic and extrahepatic BTC in view of the
mutational landscape, nearly representing 2 different tumor entities. This differences are also apparent in the subgroup
analysis of the gemcitabine/cisplatin trial by Valle et al., favoring intrahepatic BTC but still being significantly
effective in both intra- and extrahepatic BTC compared to single gemcitabine. For several mutations inhibitors are available, although none of them has proven effectivity in phase III trial setting in BTC so far. Moreover, the NIFE trial aims to widen the field of first-line Treatment options (so far gem/cis and optionally cis can be removed by oxaliplatin) due to various reasons starting with toxicity, side effects but also to have an alternative backbone for the combination with targeted therapies that may be of value in terms of efficacy. Within the discussion it is stated that the NIFE trial is accompanied by an expanded liquid biopsy project. Deliberately we will not further specify this, due to no pre-defined planned analysis in the protocol.

Reviewer 2: In this study protocol, Perkhofer L et al. designed an open label, non-comparative, randomized
and multicenter phase II study to evaluate the potential of a nonoliposomal-irinotecan with 5-fluouracil
and leucovorin in advanced biliary tract cancer.
This study protocol paper is well-written. There is no comment to improve the quality of this paper, and I
think this paper is now judge to be "accept" for BMC cancer.
Answer: We thank for this positive feedback.

Reviewer 3: This is a well written protocol paper.
I am including some comments which may assist the authors in improving their manuscript.
Keywords:
Alter cholangiocellular carcinoma to "cholangiocarcinoma" which would be better recognised.
Answer: Thanks for the suggestion, we changed to cholangiocarcinoma which might improve visibility.
Background:
Within first sentence, please clarify what is meant by "ranks beyond 10th in Western World tumor statistics". Do you mean ranked 10th in world incidence?
Answer: To specify the sentence was changed to: “Biliary tract cancer (BTC) is a rare type of cancer and ranks beyond 10th in Western World tumor incidence.”

Study objectives:
Provide Eisenhauer et al, Eur J Cancer reference following mention of RECIST 1.1.
Answer: The reference is included into the manuscript.
Provide version number of CTCAE criteria that is being used within trial.
Answer: The version number was included into manuscript. The version used is 4.03.
Within "Exploratory objectives section", please alter English to (cfDNA exome sequencing, transcriptome, miRNA-arrays prior to and after start of treatment, and on progression).
Answer: The changes were directly implemented.

Treatment:
It is mentioned that "if one drug has to be discontinued permanently under therapy for a reason other than progressive disease, treatment should continue with the remaining drug in the trial…". What if the gemcitabine has to be discontinued in the Cis/Gem arm, would you continue with cisplatin alone? This would not be usual.
Answer: Thanks for highlighting this crucial point. Within the trial the physicians can continue with either gemcitabine Arm B or 5-FU Arm A monotherapy. It’s not recommended to use the experimental drug nal-IRI or cisplatin as monotherapy. This is now specified in the manuscript.

Figure 1:
How was cut-off for age stratification determined? A potential reference that could be used would be "McNamara MG et al 2017, BMC Cancer 17: 262.
Answer: Thanks for naming the reference, while preparing the manuscript we must have overlooked it. As underlining the rationale for stratification the reference was added to the manuscript (page 5, line 33). We hope that all comments raised by the reviewers are answered to their satisfaction and the manuscript is suitable for publication in BMC Cancer. We are thankful for the reviewers and their suggestions that to our opinion improved the manuscript.

With kind regards,

Lukas Perkhofer