Dear Reviewers, dear editorial board members,

thank you very much for the kind reviewing of our manuscript, your important questions and your helpful suggestions. Answers are listed below and revisions highlighted within the manuscript. In addition we have supplemented the ARTICLE HIGHLIGHTS and adapted the subcategories of the abstract with regard to the word limitations.

1. Please tell me the reason why Podoplanin expressed squamous cell carcinoma of esophagus and did not express adenocarcinoma of the esophagus. Moreover, tell me the difference of carcinogenesis of squamous cell carcinoma and adenocarcinoma of esophagus.

Esophageal cancer comprises two epidemiologically and pathologically distinct diseases that share an anatomical site, but have divergent risk factors and incidence trends.

EAC (Esophageal adenocarcinoma) primarily develops from Barrett esophagus, a preneoplastic tissue in which the squamous esophageal epithelium is replaced by a columnar intestinal-type mucosa. EAC predominantly relies on central or visceral obesity and acid or bile reflux. This metaplasia, called Barrett esophagus, is located more common in the distal oesophagus.

Common genes mutated in EAC and ESCC, are TP53 and PIK3CA. Examples for genes more frequently mutated in EAC are SMAD4 and TLR4.

ESCC (Esophageal squamous cell carcinoma) develops from the squamous epithelial cells that makes up the inner lining of the esophagus and predominantly relies on smoking and alcohol abusus as risk factors. ESCC is located more common in the upper and middle third of the esophagous. ESCC develops from basal cell hyperplasia and dysplasia (low to high grade) to carcinoma *in situ.* NOTCH1, ZNF750 are examples for genes more frequently mutated in ESCC.

Podoplanin is also expressed in the membrane of some adult normal cells with epithelial differentiation such as the basal squamous epithelium of cervix and esophagus. This might be the reason why podoplanin is expressed at high level in ESCC, but not in EAC, since the tumor cells develop from glandular metaplasia instead of the original squamous mucosa.

Many thanks for this important question. We have included the aspect into the discussion part as follows: Discussion page 20: A reason for high podoplanin expression in squamous cell carcinoma in contrast to adenocarcinoma might be that podoplanin is expressed in some adult normal cells like lung alveolar cells, glomerular podocytes, as well as in basal epithelium of cervix and esophagus. ESCC develops from squamous epithelial cells, whereas in EAC these cells are replaced by columnar intestinal-type mucosa. EAC develops from Barrett esophagus. (Reference 27) of the manuscript: G Chen, R Xu, B Yue, X Mei, P Li, X Zhou, S Huang, L Gong, S Zhang. The expression of podoplanin protein is a diagnostic marker to distinguish the early Infiltration of esophageal squamous cell carcinoma. Oncotarget 2017, 8:19013-19020.

2. According to author's data about Podoplanin scale, score 1 is 0-5%, score 2:6-35% and score 3: 36-65%. Please tell me the reason why stronger podoplanin protein expression is >5%.

We have chosen this cut-off, since up to 5 % there is no or only weak podoplanin expression. An expression > 5% describes podoplanin staining that is clearly more than weak background or signals in some single cells.

3. According to Table 3, podoplanin did not show any significant relation to pT-category and lymph node metastasis. But Sonali Pradhau et al (Journal of applied oral science 2019) reported that the expression of podoplanin was associated with the degree of differentiation of the tumors. Please tell me the reason why podoplanin did not show any significant relation to pT-category and lymph node metastasis.

It is known that expression of podoplanin by cancer cells increases their migration and invasion capabilities. Pradhau et al 2019 described an association of the expression of podoplanin with the degree of differentiation of oral squamous cell carcinoma. Since most of our patients show locally advanced ESCC, a weakness of the correlation in our study is the low number of patients with superficial tumor stages such as pT1 or pT2 category. However, this reflects the actual clinical situation, since most patients are diagnosed at this late time point. This might explain why we were only able to show a trend instead of a significance for this aspect (as described in discussion page 22).

4**. Please tell the tailored therapy for advanced esophageal cancer about Podoplanin.**

A tailored therapy based on detection of strong podoplanin expression could result from antibodies against this membrane protein with the aim to target cancer cells of ESCC. Thus, there could be a test performed with ESCC biopsies for strong podoplanin expression with the consequence of a targeted antibody therapy against podoplanin expressing cells. Monoclonal antibodies against podoplanin have already been produced. The limitations, however, are the toxic side effects, since this protein also is present in some normal adult cells as mentioned above. One anti-human podoplanin antibody (NZ-1) inhibiting podoplanin-induced platelet aggregation, exhibits strong toxic side effects. Another example is a chimeric humanized anti-human podoplanin antibody inhibiting podoplanin-induced platelet aggregation, also interfering with endogenous podoplanin in other cell types like lung alveolar cells, kidney podocytes. Further antibodies have to be developed within the approach for a potential novel anticancer agent. More knowledge about podoplanin function has to be generated in order to minimize side effects.

UNIKLINIK KÖLN
Klinik und Poliklinik für Allgemein-, Viszeral- und Tumorchirurgie

Zertifiziertes Exzellenz-Zentrum für die Chirurgie des Magens und der Speiseröhre
DKG Zertifiziertes Onkologisches Zentrum
DKG Zertifiziertes Darmkrebszentrum
DKG Zertifiziertes Pankreaskarzinom Zentrum

Direktorin:
Univ.-Prof. Dr. med. C.J. Bruns

+Uniklinik Köln | Klinik und Poliklinik für Allgemein-, Viszeral- und Tumorchirurgie | 50937 Köln, Kerpener Str. 62

Prof. Dr. rer. nat. Ute Warnecke-Eberz
Labor für Molekulare Onkologie
Telefon: +49221 478-6011
Telefax: +49221 478-7460
Ute.Warnecke-Eberz@uk-koeln.de

Editorial Board
World Journal of Gastroenterology

Cologne, 20/04/2020

Invited Manuscript ID 0057699

Dear Editorial Board members,

thank you very much for the kind reviewing of our manuscript No53441 „**Neoadjuvant chemoradiation changes podoplanin expression in esophageal cancer patients“**, and your questions to items which we have in part already included into the manuscript within the revision process.

1. **Please tell me the reason why podoplanin is overexpressed in ESCC, but is not expressed in EAC.**

The reason could be that EAC and ESCC are epidemiologically and pathologically distinct diseases that share an anatomical site, but have divergent risk factors and incidence trends.

The tumorigenesis is different. EAC (Esophageal adenocarcinoma) primarily develops from Barrett esophagus, a preneoplastic tissue in which the squamous esophageal epithelium is replaced by a columnar intestinal-type mucosa. EAC predominantly relies on central or visceral obesity and acid or bile reflux. This metaplasia, called Barrett esophagus, is located more common in the distal oesophagus.

ESCC (Esophageal squamous cell carcinoma) develops from the squamous epithelial cells that makes up the inner lining of the esophagus and predominantly relies on smoking and alcohol abusus as risk factors. ESCC is located more commonly in the upper and middle third of the esophagous. ESCC develops from basal cell hyperplasia and dysplasia (low to high grade) to carcinoma *in situ.*

NOTCH1, ZNF750 are examples for genes more frequently mutated in ESCC. Common genes mutated in EAC and ESCC, are TP53 and PIK3CA. Examples for genes more frequently mutated in EAC are SMAD4 and TLR4.

A major reason could be the fact that podoplanin is also expressed in the membrane of some adult normal cells with epithelial differentiation such as the basal squamous epithelium of cervix and esophagus. This might be the reason why podoplanin is expressed at high level after malignant transformation in ESCC, but not in EAC, since the tumor cells of EAC develop from glandular metaplasia instead of the original squamous mucosa.

This aspect has been addressed to within the manuscript discussion page 15: „A reason for high podoplanin expression in squamous cell carcinoma in contrast to adenocarcinoma might be that podoplanin is expressed in some adult normal cells like lung alveolar cells, glomerular podocytes, as well as in basal epithelium of cervix and esophagus. ESCC develops from squamous epithelial cells, whereas in EAC these cells are replaced by columnar intestinal-type mucosa. EAC develops from Barrett esophagus. (Reference 27) of the manuscript: G Chen, R Xu, B Yue, X Mei, P Li, X Zhou, S Huang, L Gong, S Zhang. The expression of podoplanin protein is a diagnostic marker to distinguish the early Infiltration of esophageal squamous cell carcinoma. Oncotarget 2017, 8:19013-19020."

2. Please tell me criteria of the cut off point in podoplanin, low (0-5%), high (>5).

We have chosen this cut-off, since up to 5 % there is no or only weak podoplanin expression. An expression > 5% describes podoplanin staining that is clearly more than weak background or signals only in some single cells.

3. Please tell the etiology which chemoradiation reduced the level of podoplanin.

There is no data on the relation between chemoradiation and podoplanin expression. This will be the content of future studies. It has also to be investigated whether podoplanin is a direct target of the therapy or a result or secondary effect arising from chemoradiation.or an alteration in upstream single pathways.

4. Please tell me the result of chemoradiation in ESCC. How about good response, no change and bad response?

Histopathologic response classification (Puetz K *et al* 2019) in Methods of the manuscript page 7

The degree of histomorphologic regression of the primary tumor was classified into four categories (Cologne Regression Scale) by an experienced staff pathologists:

Major Response:
grade 1, complete response (no more viable tumor cells detectable within histopathologic work-up)
and grade 2, nearly complete response with less than 10% vital residual tumor cells (VRTCs)

Minor Response:
grade 3, 10% to 50% VRTCs
and grade 4, more than 50% VRTCs,

Patients, progredient during therapy have not been included into the study.

Generally, the multimodal treatment of chemoradiation within ESCC is performed with the aim to improve the patients' postsurgical prognosis (Puetz K *et al* 2019, van Hagen *et al.*, 2012). The neoadjuvant chemoradiation followed by surgery has improved patients' survival resulting in a median overall survival of 82 months compared to 21 months performing surgery alone (Shapiro *et al.*, 2015), however, only for responders of the therapy. There are still about 35% of patients without major response to chemoradiation that do not benefit by better survival (den Bakker *et al.*, 2017).

5**. According to author's data, high level of podoplanin is poor prognosis. I suspect podoplanin inhibitor is good effect for the prognosis of ESCC. Please comment about podoplanin inhibitor.**

A tailored therapy based on detection of strong podoplanin expression could result from antibodies against this membrane protein with the aim to target cancer cells of ESCC. Thus, there could be a test performed with ESCC biopsies for strong podoplanin expression with the consequence of a targeted

antibody therapy against podoplanin expressing cells. Monoclonal antibodies against podoplanin have already been produced. The limitations, however, are the toxic side effects, since this protein is also present in some normal adult cells as mentioned above. One anti-human podoplanin antibody (NZ-1) inhibiting podoplanin-induced platelet aggregation, exhibits strong toxic side effects. Another example is a chimeric humanized anti-human podoplanin antibody inhibiting podoplanin-induced platelet aggregation, also interfering with endogenous podoplanin in other cell types like lung alveolar cells, kidney podocytes. Further antibodies have to be developed within the approach for a potential novel anticancer agent. More knowledge about podoplanin function has to be generated in order to minimize the side effects.(in Discussion page 16)

Thank you very much for reviewing our manuscript. We will be readily prepared to answer all further questions resulting from this research article.

Sincerely yours,

Prof. Dr. Ute Warnecke-Eberz