22 January, 2021

Dear *BMC Cancer* Editor

Nicola Fusco

BCAN-D-20-04763 “Prediction of distant metastatic recurrence by tumor-infiltrating lymphocytes in hormone receptor-positive breast cancer”

We greatly appreciate your invitation for us to re-submit our article. We would like to thank you for a number of comments and suggestions for improvement in our manuscript entitled **“Prediction of distant metastatic recurrence by tumor-infiltrating lymphocytes in hormone receptor-positive breast cancer”** by Takada K *et al*. We have carefully considered the referee’s comments and have made point-by-point responses as described below, and highlighted in the revised manuscript.

I hope this revised manuscript can again be considered for publication in the *BMC Cancer.*

Sincerely,

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**We have responded to the reviewer reports, as follows:**

**- For a better reading, the revised parts of the manuscript should be highlighted (NOT underlined)**

⇒ We removed the underlined corrections and highlighted them in yellow.

**- Please provide the point-by-point response to the Reviewers' comments as a separate file, not only in the form**

⇒ We provided it as "answer (BMCC) R2".

**- The quality of the micrographs should be substantially improved, as the figure legends, they are still of poor quality**

⇒ We have changed the image to a higher resolution.

**- TILs should be re-evaluated according to the TILs working group recommendations, i.e. on full sections (NOT on random fields). In the response, please highlight the changes of these results.**

⇒Since the evaluation was performed using biopsy tissue, the evaluable field of view is five to ten. Even if additional fields of view are added in cases who more than 5 fields of view can be evaluated, the average does not change. We revised the text in Method.

Specifically, the density of infiltrating lymphocytes was averaged over five random fields of the tumor stroma.

→ Specifically, the density of infiltrating lymphocytes was averaged on full sections, at least five fields, of the tumor stroma.

(Page 6 line 125)

**-** **BC abbreviations should be spelled as breast cancer across the entire manuscript**

⇒We revised them as you instructed.

human epidermal growth factor receptor 2 (HER2)-enriched breast cancer (HER2BC) and triple-negative breast cancer (TNBC)

→ human epidermal growth factor receptor 2 (HER2)-enriched breast cancer and triple-negative breast cancer

(Page 2 line 26-28)

HR+HER2-BC → HR+/HER2- breast cancer

(Page 2 line 28-29) (Page 2 line 31-32) (Page 2 line 33) (Page 2 line 41) (Page 3 line 45) (Page 3 line 64- page 4 line 65) (Page 4 line 66) (Page 4 line 79) (Page 4 line 80) (Page 5 line 88) (Page 5 line 91) (Page 7 line 141) (Page10 line 191) (Page 12 line 238) (Page 12 line 241) (Page 13 line 261) (Table 2) (Table 3) (Table 4)

human epidermal growth factor receptor 2 (HER2)-enriched breast cancer (HER2BC) and

→ human epidermal growth factor receptor 2 (HER2)-enriched breast cancer and

(Page 3 line 62-63)

HER2BC and TNBC

→ HER2-enriched breast cancer and TNBC

(Page 12 line 237)

HER2: human epidermal growth factor receptor 2, HER2BC: human epidermal growth factor receptor 2-enriched breast cancer, HR+HER2-BC: hormone receptor-positive and HER2-negative breast cancer,

→ HER2: human epidermal growth factor receptor 2, HR+/HER2- breast cancer: hormone receptor-positive and HER2-negative breast cancer,

(Page 14 line 289-290)

**- use "/" to divide ER from HER2, and never use superscript. For example: ER+/HER2- (NOT ER+HER2-BC)**

⇒We revised them as you instructed.

HR+HER2-BC → HR+/HER2- breast cancer

(Page 2 line 28-29) (Page 2 line 31-32) (Page 2 line 33) (Page 2 line 41) (Page 3 line 45) (Page 3 line 64- page 4 line 65) (Page 4 line 66) (Page 4 line 79) (Page 4 line 80) (Page 5 line 88) (Page 5 line 91) (Page 7 line 141) (Page10 line 191) (Page 12 line 238) (Page 12 line 241) (Page 13 line 261) (Table 2) (Table 3) (Table 4)

**- Please provide references for the first sentences of the introduction**

⇒Refetence 4-6 is the reference for the first sentence. Therefore, the order of references 1-6 has changed.

still has the risk of recurrence. Breast cancer often has local recurrence and axillary lymph node metastasis,

→ still has the risk of recurrence [1-3]. Breast cancer often has local recurrence and axillary lymph node metastasis,

(Page 3 line 52)

[1-3] → [4-6]

(Page 3 line 57)

[4, 5] → [1, 2]

(Page 3 line 60)

[4, 6] → [1, 3]

(Page 3 line 61)

[5, 25-27] → [2, 25-27]

(Page 10 line 207)

[5] → [2]

(Page 10 line 208)

[5, 26] → [2, 26]

(Page 10 line 209)

[4] → [1]

(Page 11 line 233)

1. Impact of follow-up testing on survival and health-related quality of life in breast cancer patients. A multicenter randomized controlled trial. The GIVIO Investigators. JAMA 1994, 271(20):1587-1592.

2. Rosselli Del Turco M, Palli D, Cariddi A, Ciatto S, Pacini P, Distante V: Intensive diagnostic follow-up after treatment of primary breast cancer. A randomized trial. National Research Council Project on Breast Cancer follow-up. JAMA 1994, 271(20):1593-1597.

3. Palli D, Russo A, Saieva C, Ciatto S, Rosselli Del Turco M, Distante V, Pacini P: Intensive vs clinical follow-up after treatment of primary breast cancer: 10-year update of a randomized trial. National Research Council Project on Breast Cancer Follow-up. JAMA 1999, 281(17):1586.

4. Chen W, Hoffmann AD, Liu H, Liu X: Organotropism: new insights into molecular mechanisms of breast cancer metastasis. NPJ Precis Oncol 2018, 2(1):4.

5. Lim YJ, Lee SW, Choi N, Kwon J, Eom KY, Kang E, Kim EK, Kim JH, Kim YJ, Kim SH et al: A Novel Prognostic Nomogram for Predicting Risks of Distant Failure in Patients with Invasive Breast Cancer Following Postoperative Adjuvant Radiotherapy. Cancer Res Treat 2018, 50(4):1140-1148.

6. Wu Q, Li J, Zhu S, Wu J, Chen C, Liu Q, Wei W, Zhang Y, Sun S: Breast cancer subtypes predict the preferential site of distant metastases: a SEER based study. Oncotarget 2017, 8(17):27990-27996.

→

1. Chen W, Hoffmann AD, Liu H, Liu X: Organotropism: new insights into molecular mechanisms of breast cancer metastasis. NPJ Precis Oncol 2018, 2(1):4.

2. Lim YJ, Lee SW, Choi N, Kwon J, Eom KY, Kang E, Kim EK, Kim JH, Kim YJ, Kim SH et al: A Novel Prognostic Nomogram for Predicting Risks of Distant Failure in Patients with Invasive Breast Cancer Following Postoperative Adjuvant Radiotherapy. Cancer Res Treat 2018, 50(4):1140-1148.

3. Wu Q, Li J, Zhu S, Wu J, Chen C, Liu Q, Wei W, Zhang Y, Sun S: Breast cancer subtypes predict the preferential site of distant metastases: a SEER based study. Oncotarget 2017, 8(17):27990-27996.

4. Impact of follow-up testing on survival and health-related quality of life in breast cancer patients. A multicenter randomized controlled trial. The GIVIO Investigators. JAMA 1994, 271(20):1587-1592.

5. Rosselli Del Turco M, Palli D, Cariddi A, Ciatto S, Pacini P, Distante V: Intensive diagnostic follow-up after treatment of primary breast cancer. A randomized trial. National Research Council Project on Breast Cancer follow-up. JAMA 1994, 271(20):1593-1597.

6. Palli D, Russo A, Saieva C, Ciatto S, Rosselli Del Turco M, Distante V, Pacini P: Intensive vs clinical follow-up after treatment of primary breast cancer: 10-year update of a randomized trial. National Research Council Project on Breast Cancer Follow-up. JAMA 1999, 281(17):1586.

**- As already required by Reviewers, please revise the entire manuscript deeply for grammar, syntax, and writing style. In this respect, you should have your manuscript reviewed by someone who is fluent in English.**

⇒ We confirmed the correction of grammar. We asked native check over the whole paper and proofread an English sentence. (Editage, No. INQ\_GIZXC\_41\_2)

**Reviewer reports:**

**Reviewer 1: In this manuscript, the authors conducted morphological assessment of tumor-infiltrating lymphocytes (TILs) in order to reveal a potential association between TIL density and recurrence site in hormone receptor-positive and HER2-negative breast cancer (HR+HER2-BC). It is demonstrated that patients with no TILs density presented less local recurrence while distant metastases were significantly higher in comparison with patients with TILs density concluding that TILs might be able to predict distant metastatic recurrence.**

**\* In the abstract, although the authors conclude that TILs may be able to predict distant metastatic recurrence in stages I- II of HR+HER2-BC, there are no findings that specifically support this conclusion decreasing the reliability of the study.**

⇒ Thank you for the suggestion. As far as we can find, there are no reports that TILs may be able to predict distant metastases in stage I- II of HR+HER2-BC. However, as described in the discussion, there are reports that the recurrence site differs depending on the TILs in early-stage TNBC and in cervical squamous cell carcinoma. The studies *in vivo* using breast cancer cell lines have also reported that immunosuppression with iTME was likely to cause distant metastasis (discussion line 247-255). We believe that these results support our research results.

**\* The total number of patients included in this study should be stated.**

⇒ Thank you for the suggestion. Although it was described in Method and Result, it is difficult to understand, so we added to Methods as below.

Of them, forty-two patients had recurrence during postoperative follow-up. They were the subject of this study.

(add to page5 line89-90)

**\* In the background, the hypothesis and the aim of the study are clearly stated. However, the authors should give greater emphasis on the distant metastatic recurrence in HR+HER2-BC patients.**

**Several points should also improved in the discussion section.**

⇒ Thank you for the suggestion. We added to the discussion why the prediction of distant metastasis is important.

When distant metastasis generates symptoms, the patient’s QOL is impaired.

→ Currently, postoperative follow-up is recommended by interview, palpation, and mammography. On the other hand, whole-body imaging for asymptomatic breast cancer patients after surgery is not recommended [4-6]. However, when distant metastasis generates symptoms, the patient’s QOL is impaired. In addition, there is a risk that treatment options may be reduced if the general condition deteriorates due to recurrence. Therefore, it is important to pick up breast cancer that is prone to distant metastasis.

(Page 13 line 273-278)

**\* The results of the study are not adequately discussed. For example, in the 2nd and 3rd paragraph they report previous studies without discussing their findings in this content.**

⇒ Thank you for the suggestion. The idea of “seed and soil” is important for cancer to form distant metastases. We have described it in the second paragraph because it is necessary to consider the molecular pathological characteristics of the cancer cells themselves, which are the seeds, and because the characteristics affect the iTME. The beginning of the second paragraph is a list of previously reported results, but those reports also suggest that it is important to influence the iTME. Since this study examined only the TILs density, the second paragraph may seem to be a little different from the concept of this study. But it is necessary from the viewpoint of “seed and soil”.

**\* Given the important limitations of this study such as the small number of cases with recurrence, the authors should explain how these constraints could be overcome.**

⇒ Thank you for the suggestion. We think it will be important to accumulate cases in the future. We also think that it is important to analyze subtypes of TILs (CD8, FOXP3, PD1, PDL1, etc.). We clarified that point.

Furthermore, since TILs also have subtypes and their functions are different, detailed elucidation of them is necessary.

(add to page 13 line 264-265)

**\* Most importantly, the authors conclude that ''This study suggested TILs to possibly be predictors of distant metastatic recurrence in stages I-II HR+HER2-BC''; however, this conclusion is not clearly supported by the results.**

⇒ Thank you for the suggestion. Certainly, there are some limits, so we think it’s an overstatement. We revised it as follows.

This study suggested TILs to possibly be predictors of distant metastatic recurrence in stages I–II of HR+HER2-BC.

→ This study suggested TILs to possibly be one of predictors of distant metastatic recurrence in stages I–II of HR+/HER2- breast cancer without chemotherapy.

(Page 14 line 283-284)

**Finally, both the figure legends should be written more explicitly.**

⇒ Thank you for the suggestion. We revised the figure legends.

**Fig. 1.** Consort diagram. A total of 709 patients with primary resectable breast cancer underwent surgery between 2007 and 2015 at the Osaka City University Hospital. Four-hundred and seventy-one patients with HR+HER2-BC underwent surgery as the first treatment and received adjuvant endocrine therapy except for adjuvant chemotherapy.

→ **Fig. 1.** Consort diagram. A total of 709 patients with primary resectable breast cancer underwent surgery between 2007 and 2015 at the Osaka City University Hospital. Two-hundred and thirty-eight patients were excluded from this study because they had non-HR+/HER2- breast cancer and/or received adjuvant chemotherapy. Four-hundred and seventy-one patients with HR+/HER2- breast cancer underwent surgery as the first treatment and received adjuvant endocrine therapy except for adjuvant chemotherapy. Since four-hundred and twenty-nine patients did not have recurrence during postoperative follow-up, fourty-two patients with recurrence were targeted in this study.

(Page 25 line 505-511)

**Fig. 2**. Histopathologic evaluation of the tumor-infiltrating lymphocytes (TILs) density was performed on hematoxylin and eosin-stained tumor section. TILs density was divided into four groups (>50% (A); >10–50% (B); ≤10% (C); and absent (D), respectively).

→ **Fig. 2**. Histopathologic evaluation of the tumor-infiltrating lymphocytes (TILs) density was performed on hematoxylin and eosin-stained tumor section. The density of them was averaged on full sections, at least five fields, of the tumor stroma. The results were divided into four groups (>50% (A); >10–50% (B); ≤10% (C); and absent (D), respectively).

(Page 25 line 513-515)

**Reviewer 2: This paper from Takada et al is an interesting paper which needs some revisions, starting with the language that needs to be improved.**

**Then there are some inconsistencies**

**Background:**

**line 65: what does it mean "local"?**

⇒We apologize for the incomprehensible description. We revised it as follows.

Therefore, local assessment of HR+HER2-BC has been suggested to be vital.

→ Therefore, early detection of locoregional recurrence after surgery for HR+HER2-BC has been suggested to be vital.

(Page 4 line 65-66)

**line 67: the sentence is not clear.**

⇒We apologize for the incomprehensible description. We revised it as follows.

Invasion and metastasis of cancer involve not only the cancer, but also the surrounding interstitial region, the so-called tumor microenvironment (TME)

→ Invasion and metastasis of cancer involve not only the molecular pathological features of the cancer itself, but also the surrounding interstitial region. The region is commonly called tumor microenvironment (TME)

(Page 4 line 68-70)

**In this Reviewer's opinion, it is not clear which is the endpoint of the study: more exactly, which is the site of metastases the Authors are referring to. Lymph node metastases or distant metastases? And amongst distant metastases, would they predict the sites?**

⇒ Thank you for the suggestion. This study shows the possibility that TILs can predict distant metastases (not locoregional recurrence) in breast cancer that met certain conditions. Since it is difficult to understand in our description, we added the following. In addition, it was not possible to predict the site of distant metastasis because the number of recurrence cases in this study was small. However, as shown in the discussion, it is also suggested that the organs that easily metastasize differ depending on the immune suppression of TME. Depending on the accumulation of future cases, analysis of subtypes of TILs or TILs alone may reveal the characteristics of metastatic organs. We think this is our future task. So, we needed to show our hypothesis before the experiment. The necessity of the experiment was added to “discussion”.

Although not known in this study, the analysis may reveal organs that are prone to metastasis.

(added to page 13 line 269-270)

In stages I–II of HR+/HER2 breast cancer, locoregional recurrence is common and distant metastasis is relatively rare. However, prediction of distant metastasis is also important for those breast cancers.

(added to page 14 line 281-282)

**Then, in this session, the Authors should argument why they think that TILs density could be correlated to site of recurrences.**

⇒ Thank you for the suggestion. It has recently been shown that the iTME affects cancer infiltration and metastasis. Among them, there are some reports on lymph node metastasis as described in “Backgrounds”. We hypothesized that if lymph node metastasis is involved, it may also be involved in distant metastasis, and conducted this study. We added to “Backgrounds”.

There are few reports on the relationship between TILs and distant metastases compared to the relationship between TILs and lymph node metastases. We speculate that if TILs are involved in lymph node metastasis, which is a risk factor for distant metastasis, TILs are also involved in distant metastasis.

(added to page 4 line 74-77)

**Methods: the Authors state that diagnosis was made on bioptic material (CNB/VAB) as well as the histological subtyping of the disease. Does it mean that even the biological characteristics (ER/PgR/Ki67/HER2) were assessed only on the biopsies? Authors should explain this issue in detail, as well was, they should explain if there was complete correspondence in all bioptic cases matched with their surgical specimens.**

⇒ Thank you for the suggestion. In this study, the biological characteristics were also evaluated in biopsy tissue. In the previous reports, it was reported that the biopsy tissue and the surgical specimen differed by about 2% at the lowest and about 20% at the higher, so caution is required (***Breast Cancer Res Treat 2012; 134(3): 957-67***). However, in the 42 cases examined in this study, the biopsy tissue and the surgical specimen were all the same, so the description was omitted. We thought it was necessary to specify it, so we added it to Methods.

The biological characteristics (ER/PgR/Ki67/HER2) of the resected specimens were examined again, and the results were all the same as those of the biopsy tissue.

(add to page 5 line 104-106)

**Why the Authors have used the 14 ref to divide the patients into high and low Ki67 rate, when Cheang et al referred to luminal B breast cancer?**

⇒ Thank you for the suggestion. In recent years, Ki67 has often set a cutoff value of 20%. However, for the following two reasons, 14% was set as the cutoff value in this study. The first reason is that the same result was shown regardless of whether the cutoff value was 14% or 20% in this study. The second reason is that there is still no consensus on a cutoff value of 20%. However, some people, like reviewer, still have doubts about setting the cutoff value at 14%, so we revised it to the cutoff value of 20%. There is no change in the analysis results.

14% → 20%

(Page 5 line 94) (Table. 1) (Table. 2) (Table. 3) (Table. 4) (Table. 5) (Table. S1) (Table. S2)

14. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS et al: **Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer.** *J Natl Cancer Inst* 2009, **101**(10):736-750.

→

14. A Goldhirsch, E P Winer, A S Coates, R D Gelber, M Piccart-Gebhart, B Thürlimann, H-J Senn, Panel members: **Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013**. *Ann Oncol 2013*; **24**(9): 2206-23.

(Reference14)

**Moreover, the Authors are encouraged to indicate which edition of TNM has been used. In table 1 micrometastatic diseases are staged as "pT1mic", which is not currently the correct way to stage those diseases.**

⇒ Thank you for the suggestion. Since the TNM classification is described, Methods and Table were revised. Subsequent references have changed.

The progression of cancer was evaluated by the 8th edition of the TNM classification of malignant tumors (UICC-TNM) by the Union for International Cancer Control (UICC) [15].

(added to page 5 line 95-97)

15. Cserni G, Chmielik E, Cserni B, Tot T: **The new TNM-based staging of breast cancer**. *Virchows Arch* 2018; **472**(5): 697-703.

(added to reference.15)

pN1mic → pN1mi

(Table. 1) (Table. 2) (Table. 3) (Table. 4) (Table. 5) (Table. S1) (Table. S2)

**Finally, there are no piece of information about the way the Authors have collected the data. This reviewer means if the histological slides have been diagnosed by one of more pathologists or if the Authors have simply extracted the data from the reports.**

⇒ Thank you for the suggestion. We asked two breast pathologists to evaluate TILs density and confirmed that there were no mistakes. Since it was re-evaluated, we added the fact to Methods and acknowledgement.

The pathological diagnosis and examination were jointly performed by two breast pathologists.

(added to page 6 line 115-116)

Acknowledgements

We thank Sayaka Tanaka and Masahiko Ohsawa (Department of Diagnostic Pathology, Osaka City University Graduate School of Medicine) for helpful advice regarding pathological diagnosis.

(added to page 16 line 320-321)

**Results: it is declared that the cohort of patients enrolled int the study excluded patients treated with chemotherapy; nevertheless, there were some patients who had axillary clearance because of metastatic disease in the sentinel lymph node. It is not clear why these pattens with axillary dissection and further metastases were not treated with chemotherapy. It could depend on the age, but the Authors should clarify.**

⇒ Thank you for the suggestion. The breast cancer patients with lymph node metastases were slightly older but had no apparent significant difference. Older age, patient refusal, comorbidities may be the causes of not taking chemotherapy. However, the obvious cause of not receiving chemotherapy was unknown because this study is retrospectively examined and some of them are judged by the attending physician. We added this point.

Whether to receive adjuvant chemotherapy was decided at the discretion of the attending physician, taking into consideration the patient's wishes and comorbidities.

(added page 5 line 106- page 6 line 108)

**How did the Authors differentiate the venous from lymphatic tumoral invasion? How the Authors explain this high percentage (50%) of vascular invasion with the low rate of metastatic disease?**

⇒ Thank you for the suggestion. If the evaluation was difficult, it was evaluated with immunostaining. We speculated that lymphatic invasion and/or vascular invasion affect metastasis, but this study did not clarify the cause. The exact cause was unknown. One of the causes might be that only the cases of recurrence were targeted. We added to limitation.

It may also be a limitation that younger and lymphovascular invasion, which have been reported as risk factors in the previous report, were not predictors in this study.

(added to page 13 line 265-267)

**lines 146-149: it is not clear the distribution of the recurrences. Please, the Authors should rephrase the sentence.**

⇒We apologize for the incomprehensible description. We revised it as follows.

Most of the first recurrence sites had locoregional recurrence (local recurrence: 18 patients (42.9%), regional lymph node: 13 patients (30.9%)). Eleven patients (26.2%) had distant metastases at the first recurrence site, 7 (16.6%) of which were lung metastases.

→ The first recurrence was local in eighteen patients (42.9%). It was the regional lymph node in thirteen patients (30.9%). Eleven patients (26.2%) had distant metastases at the first recurrence site. Regarding distant metastases, seven patients (16.6%) had lung metastases, which was the most common of the distant metastases. No case had locoregional recurrence and distant metastasis at the same time.

(Page 8 line 158-162)

**It is not understandable for this Reviewer why the Authors have used a nuclear grade. Please, Authors explain this issue.**

⇒ Thank you for the suggestion. Both histological grade and nuclear grade are predictors of recurrence, but nuclear grade is one of the factors that influence TILs. Therefore, we made NG one of the items to be considered.

**Discussion: the Authors claim that operative procedures are predictive or recurrences, but do not explain the reasons (lines 195-6).**

⇒ Thank you for the suggestion. As shown in Table S1, the “BCT and radiation therapy” group was significantly younger and had a higher Ki67 than the “mastectomy” group (Result line 171-175). It has been reported that they are recurrence factors for distant metastasis (Discussion line 206). In this study, it did not become a risk as a single factor, but we thought that it became a risk factor when combined. it was described in Discussion, but it is difficult to understand, so we revised.

In this study, operative procedure was shown to be a predictive factor for distant metastasis, and the cause was found to be correlated with the operative procedure as well as some clinicopathological features, such as age, tumor diameter, and Ki67, also referred to as risk factors.

→ In this study, choosing “BCT and radiation therapy” for operative procedure was shown to be a predictive factor for distant metastasis. The cause was that the “BCT and radiation therapy” group was significantly younger, larger tumor diameter and had a higher Ki67 than the “mastectomy” group. They are the risk factors listed above. Each item alone did not become a predictor, but it became a predictor when combined.

(Page 10 line 209-page11 line 214)

**In this Reviewer's opinion, the item regarding the molecular characterization of tumor cells is not pertinently introduced at this point without any hints in the previous sections.**

⇒ Thank you for the suggestion. The idea of “seed and soil” is important for cancer to form distant metastases. We have described it in the second paragraph because it is necessary to consider the molecular pathological characteristics of the cancer cells themselves, which are the seeds, and because the characteristics affect the iTME. The beginning of the second paragraph is a list of previously reported results, but those reports also suggest that it is important to influence the iTME. Since this study examined only the TILs density, the second paragraph may seem to be a little different from the concept of this study. But it is necessary from the viewpoint of “seed and soil”.

**Finally, Authors claimed and claim that "…TILs may also be involved in lymph node metastasis…" in Luminal cancer. Nevertheless, the results indicate that they correlate with venous invasion (p=0.007) which is already related to metastatic diseases.**

⇒ Thank you for the suggestion. Certainly, there was a correlation between TILs and venous invation, but this was when the cutoff value for TILs was set to 10%. In the results of this study, absent of TILs was correlated with the recurrence of distant metastases. We added to discussion.

It may also be a limitation that younger and lymphovascular invasion, which have been reported as risk factors in the previous report, were not predictors in this study. It is also possible that TILs are involved in venous invasion.

(added to page 13 line 265-268)