

## ANSWERING REVIEWERS



December 20, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: .doc).

**Title:** Targeting receptor tyrosine kinases in gastric cancer.

**Author:** Asahiro Morishita, Gong Jian, Tsutomu Masaki

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 6820

The manuscript has been improved according to the suggestions of reviewers:

**1. Format has been updated**

**2. Revision has been made according to the suggestions of the reviewer 00608332.**

*(1) Figure 2 does not seem to add to the already published article (ref. 22) and could be removed.*

We apologize for the duplication of figure 2 and we remove it in revised manuscript.

*(2) The authors could choose to provide a schematic representation of RTKs with increased expression in AGC, but its message would be similar to that of figure 1.*

We modified figure 1 to emphasize the up-regulated RTKs in AGC, such as HER-1, HER-2, IGF-1R and FGFR using red font.

*(3) Abbreviations are missing for most genes on first appearance in the text.*

We amended abbreviations of most genes which are indicated on first appearance and used them consistently afterwards.

*(4) Does the sentence "The survival rate of gastric cancer has remained at 20-30% over the past 35 years. (page 4, line 9)" refers to a 5-year interval?*

Reviewer is correct. We added '5-year' in front of survival rate and replaced the words from '30%' and "over the past 35 years" to '25%' and "in the western world".

*(5) The sentences "Receptor tyrosine kinases (RTKs) consist of/are characterized by a transmembrane and tyrosine kinase domain..." (pages 4 and 6) have to refer to the existence of an extracellular domains, that are the regions that identify the subfamilies of RTKs.*

We added the part of the sentence 'of ligand-binding extracellular domains which identify the subfamilies of RTKs' between "Receptor tyrosine kinases (RTKs) consist" and "a transmembrane and tyrosine kinase domain..." (pages 4 and 6).

(6) *In page 9 there are several instances of words/sentences written in a different letter size.*

We replaced the several words of different letter size to correct letter size.

(7) *Despite the extense list of references, the rapidly growing literature adds new studies every week. Althouth it is impossible to include them all, the authors should consider to include the recent developments on the use of Trastuzumab:Singh R,Kim WJ, Kim PH, Hong HJ. Exp Mol Med. 2013 Nov 1;45:e52. doi: 10.1038/emm.2013.111.and Qiu MZ, Li Q, Wang ZQ, Liu Ts.....*

We added new reference; "Recently, Singh et al. reported combined blickeade of HER2 and VEGF brings about greater growth inhibition in HER-2 overexpressing gastric cancer xenografts (Singh et al.). This result suggests that new combination therapy using inhibitors of HER-2 and VEGF may represent a new approach for the treatment of HER-2 positive AGC patients." in page 16.

(8) *Page 6. "metabolism pathways" should be "metabolic pathways".*

We altered the word from "metabolism pathways" to "metabolic pathways".

(9) *Page 7. "which are characterized by similar structures and the potential for intrafamilial dimerization in gastric cancer"*

We modified the sentence from "which are characterized by similar structures and the potential for intrafamilial dimerization in gastric cancer" to "which are characterized by similar structures and the potential of dimerization in gastric cancer".

(10)*"It has been reported that common alterations and mutations that may serve as targets for molecularly targeted treatments have been identified in gastric cancer." Not clear In page 14".*

We modified the sentence from "It has been reported that common alterations and mutations that may serve as targets for molecularly targeted treatments have been identified in gastric cancer." to "Common alterations and mutations of RTKs have been identified in gastric cancer."

(11)*"Regarding adverse events, grade 4 cardiac toxicity and vomiting were observed in 47 patients with metastatic gastric cancer" has to provide %.*

We altered the sentence from "Regarding adverse events, grade 4 cardiac toxicity and vomiting were observed in 47 patients with metastatic gastric cancer" to "Regarding adverse events, one patient each experienced grade 4 cardiac toxicity and vomiting in 47 patients with metastatic gastric cancer".

### 3. Revision has been made according to the suggestions of the reviewer 02769326.

(1) *Figure 2 appears to be a duplicate of that already found in the literature previously published by this group.*

We apologize for the duplication of figure 2 and we remove it in revised manuscript.

(2) *Need to be addressed is a more complete and up to date review of other RTKs involved in gastric carcinoma (for instance the role of the TAM RTK family (Tyro3, Axl, MerTK) in gastric carcinoma has been multiply described).*

We added one paragragh in the section of "RTKs in gastric cancer" in page 7 to address the explanation

of TAM RTK family and gastric carcinoma.

(3) *Please define all of the abbreviations in the 'Core Tips' section and throughout the paper.*

We amended abbreviations of most genes which are indicated on first appearance and used them consistently afterwards.

(4) *Core tips section needs some grammatical corrections.*

We modified the some grammatical errors.

(5) *What makes Receptor TKs different specifically is that they contain an extracellular portion, this should be clarified as only the transmembrane and kinase motif are mentioned.*

We added the part of the sentence 'of ligand-binding extracellular domains which identify the subfamilies of RTKs' between "Receptor tyrosine kinases (RTKs) consist" and "a transmembrane and tyrosine kinase domain..." (pages 4 and 6).

(6) *Intro section should use generic instead of brand names for the therapeutics since the brand names vary by country of marketing.*

All brand names, such as Herceptin, Gleevec, Iressa, and Erbitux, were modified to generic names, such as trastuxumab, imatinib, gefitinib, and cetuximab in introduction section.

(7) *'RTKs in cellular signaling' section is incomplete. There is no discussion of the normal functions of RTKs, ie they are necessary for a variety of normal cellular physiologic processes. And in many cases are NORMALLY present. The implication from this section is that they are only present in malignant cells.*

We added and amended the sentences in page6 from "In various types of cancer, many signaling pathways including cell proliferation, differentiation, and metabolism pathways, are activated by RTK dimerization<sup>[5, 16]</sup>." to "Various RTKs have been normally associated with intracellular signal transduction including growth, differentiation, adhesion, migration, and apoptosis (Hubbard and Till). In various types of cancer, many signaling pathways including cell proliferation, differentiation, and metabolism pathways, are activated by RTK dimerization<sup>[5, 16]</sup>."

(8) *The authors refer to the use of RTK inhibition in multiple clinical trials and then cite clinical trials that do not target RTKs, ie imatinib in the case of CML, while imatinib does target the KIT RTK in GIST. Again confusing the concept of targeting RECEPTOR TLs versus Just TKs.*

Reviewer is correct. We deleted the part; "imatinib for chronic myelogenous leukemia and gastrointestinal stromal tumors" from page 5.

(9) *In the 'RTKs in gastric cancer' section, the authors discuss the 21 families of RTKs, and discuss the 'potential for intrafamilial dimerization in gastric cancer', however this potential is there in normal settings and perhaps physiologically relevant for normal function, this should be clarified.*

We described this answer in the part of (7).

(10) *There needs to be some commentary on how each of the RTKs introduced in the manuscript*

*differs and how each RTK inhibitor works, and in general that there is more than one way to target an RTK. Ie inhibiting/decreasing receptor expression, sequestering ligand, and or directly inhibiting the ATP binding activation site.*

We added the sentences, "Each RTK inhibitor is diagramed in Figure 1 and inactivates various RTKs. Among these RTK inhibitors, monoclonal antibodies, such as cetuximab and panitumumab, trastuzumab and fugitumumab directly bind to each RTK and inhibit its signaling. Bevacizumab also binds VEGF and inhibit VEGFR signaling. Gefitinib and erlotinib competes with the binding of ATP to the tyrosine kinase domain of EGFR and lapatinib blocks phosphorylation of HER-1 and HER-2. Sorafenib inhibits the enzyme RAF kinase and VEGFR-2/PDGFR-beta signaling cascade. Sunitinib also blocks VEGFR-2/PDGFR-beta and c-kit. In addition, GSK089 inhibits c-Met and blocks its signaling." in page 7.

*(11)The 'RTKs in gastric cancer' section needs to be more complete. The explanation of the molecular biology and intracellular signaling of RTKs relies on studies conducted 15 to 20 years ago (References 16-18, 20)... the significance fo the TAM (aka Axl family) RTK family in gastric carcinoma prognosis and several studies that inhibit it with some efficacy. These should be discussed for completeness*

We described this answer in the part of (2).

*(12)There may be others to be discussed. Anticancr Res. 2002...Mol Carcinog. 2007.....*

We added one paragraph; "In addition, AXL receptor tyrosine-kinase family, such as axl/ufo and nyk/mer protein kinases, co-operatively correlates with the cancer progression, metastasis and patients' prognosis in gastric cancer (Wu CW, Li AF). The specific ligand growth arrest-specific gene 6 (Gas6) binds to Axl and Gas6-Axl signaling pathway enhanced cellular survival and invasion and suppressed apoptosis via Akt family during gastric carcinogenesis (Sawabu t, Seno h). Gas6-Axl signaling could be a potential therapeutic target in gastric cancer." into page 16.

#### 4 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



Asahiro Morishita, MD, PhD  
Department of Gastroenterology and Neurology  
Kagawa University School of Medicine  
1750-1 Ikenobe, Miki-cho, Kida-gun, Kagawa, Japan  
Tel: +81-87-891-2156  
Fax: +81-87-891-2158  
E-mail: asahiro@med.kagawa-u.ac.jp

