Author's response to reviews

Title: Assessment of early changes in 3H-fluorothymidine uptake after treatment with gefitinib in human tumor xenograft in comparison with Ki-67 and phospho-EGFR expression

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Author's response to reviews: see over
ReRe: BMC Cancer – MS: 5717212398910526

Title: $^3$H-fluorothymidine can be used for early and accurate monitoring of antiproliferative effect of gefitinib in human tumor xenograft: Comparison with Ki-67 and phospho-EGFR expression

Authors: Songji Zhao et al.

Dear Dr. Solera:

We have re-revised our manuscript (MS: 5717212398910526) entitled “$^3$H-fluorothymidine can be used for early and accurate monitoring of antiproliferative effect of gefitinib in human tumor xenograft: Comparison with Ki-67 and phospho-EGFR expression” in accordance with the comments and suggestions of the reviewers. On a separate sheet, we have provided our point-by-point responses to the comments and suggestions made by the reviewers. Red text was used to indicate all the changes within the manuscript itself, and deletions have been noted in our point-by-point replies.

We look forward to the publication of our manuscript in BMC Cancer.

Sincerely yours,

Yuji Kuge, Ph.D.
On behalf of all the authors

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We are grateful to the reviewers again for their insightful comments and useful suggestions that helped us improve our manuscript. As indicated in the responses that follow, we have taken all of the comments and suggestions into account in preparing the re-revised version of our manuscript.

Our point-by-point responses are written below each of the reviewer’s comments.

Reviewer #1:

I have no further comments on this revised manuscript. The authors responded adequate on all comments I gave in my first review of the manuscript.

Response:

We again thank the reviewer for their useful comments.

Reviewer #2

I would like to thank the authors for detailed response. I believe the authors have been very thoroughly in their response to prior review. However, I have some additional comments that need to be addressed.

Major Compulsory Revisions
1) The title still does not describe the content of this study. First, [3H]fluorothymidine is not an imaging agent to be used for monitoring the effect of gefitinib. In fact, [3H]fluorothymidine cannot be used for monitoring. Second, “accurate” monitoring of antiproliferative effect may not be assessed in a single xenograft model. This study assessed early changes in [3H]fluorothymidine uptake after gefitinib in human tumor xenograft in comparison with Ki-67 and phosphor-EGFR expression.
Response:
We thank the reviewer for the useful comment and completely agree with this point. In accordance with the reviewer’s suggestion, we have revised the title:

Corrections:
From: “3H-fluorothymidine can be used for early and accurate monitoring of antiproliferative effect of gefitinib in human tumor xenograft: Comparison with Ki-67 and phospho-EGFR expression”
To: “Assessment of early changes in 3H-fluorothymidine uptake after treatment with gefitinib in human tumor xenograft in comparison with Ki-67 and phospho-EGFR expression”

2) In the same context, the sentences including “can be used” and “usefulness” in the abstract, introduction and discussion section should be reworded.

Response:
We completely agree with the reviewer and we have accordingly reworded the sentences including “can be used” and “usefulness” in the abstract, introduction, and discussion in the revised manuscript as follows:

Corrections:
(1) Page 3, lines 2 to 5 in the background of the “Abstract” section:
From: “Background: The purpose of this study was to evaluate whether 3’-deoxy-3’-3H-fluorothymidine (3H-FLT) can be used for the early and accurate detection of the antiproliferative effect of gefitinib in a human tumor xenograft, in comparison with the histopathological markers, Ki-67 and phosphorylated EGFR (phospho-EGFR).”
To: “Background: The purpose of this study was to evaluate whether early changes in 3’-deoxy-3’-3H-fluorothymidine (3H-FLT) uptake can reflect the antiproliferative effect of gefitinib in a human tumor xenograft, in comparison with the histopathological markers, Ki-67 and phosphorylated EGFR (phospho-EGFR).”

(2) Page 4, lines 13 to 15 in the conclusion of the “Abstract” section:
From: “Thus, it was indicated that 3H-FLT as a surrogate biomarker can be used for monitoring the antiproliferative effect of gefitinib in a mouse model of a human epidermoid cancer.”
To: Thus, it was indicated that **early changes in \(^3\)H-FLT uptake may reflect** the antiproliferative effect of gefitinib in a mouse model of a human epidermoid cancer.”

(3) Page 8, lines 12 to 13 in the “Background” section:
From: “Sohn et al. demonstrated that \(^{18}\)F-FLT PET **can be used to** predict early responses to gefitinib treatment in patients with advanced pulmonary adenocarcinoma [25].”
To: Sohn et al. demonstrated that \(^{18}\)F-FLT PET **can predict** early responses to gefitinib treatment in patients with advanced pulmonary adenocarcinoma [25].”

(4) Page 8, line 15 to page 9, 1 in the “Background” section:
From: “Although, several studies have indicated the **usefulness of** \(^{18}\)F-FLT or \(^3\)H-FLT for monitoring** effect of gefitinib [25,26], whether **\(^3\)H-FLT as a surrogate biomarker can be used for accurately monitoring** the effect of gefitinib by comparing the level of \(^3\)H-FLT uptake with those of other proliferation or predictive markers, such as Ki-67 or phosphorylated EGFR, in an early phase of treatment has not been fully validated under a pathological condition.”
To: “Although several studies have indicated the **ability of** \(^{18}\)F-FLT or \(^3\)H-FLT to detect the** effect of gefitinib [25,26], whether **changes in \(^{18}\)F-FLT uptake can reflect** the effect of gefitinib by comparing the level of \(^{18}\)F-FLT uptake with those of other proliferation or predictive markers, such as Ki-67 or phosphorylated EGFR, in an early phase of treatment has not been fully validated under a pathological condition.”

(5) Page 9, lines 2 to 6 in the “Background” section:
From: “Thus, in the present study, to evaluate whether **\(^3\)H-FLT as a surrogate biomarker can be used for the early and accurate detection of** the antiproliferative effect of gefitinib, we determined the changes in \(^3\)HFLT uptake level after the start of treatment at different doses of gefitinib in comparison with those in \(^{18}\)F-FDG uptake, Ki-67 expression, and phospho-EGFR levels in a human tumor xenograft (EGFR-dependent human tumor xenograft model, A431).”
To: “Thus, in the present study, to **determine** whether **early changes in \(^3\)H-FLT uptake can reflect** the antiproliferative effect of gefitinib, we determined the changes in \(^3\)HFLT uptake level after the start of treatment at different doses of gefitinib in comparison with those in \(^{18}\)F-FDG uptake, Ki-67 expression, and phospho-EGFR levels in a human tumor xenograft (EGFR-dependent human tumor xenograft model, A431).”
(6) Page 20, lines 8 to 10 in the “Discussion” section:
From: “Our present findings suggest that \(^{18}\text{F-FLT}\) can predict the therapeutic effect of gefitinib at a very early time point (2-days after the start of gefitinib treatment) during which changes in tumor size cannot be detected yet.”
To: “Our present findings suggest that \(^{3}\text{H-FLT}\) can predict the therapeutic effect of gefitinib at a very early time point (2-days after the start of gefitinib treatment) during which changes in tumor size cannot be detected yet.”

(7) Page 22, lines 16 to 18 in the “Discussion” section:
From: “Thus, it was indicated that \(^{3}\text{H-FLT}\) as a surrogate biomarker can be used for monitoring the antiproliferative effect of gefitinib was indicated in a mouse model of human epidermoid cancer.”
To: “Thus, it was indicated that early changes in \(^{3}\text{H-FLT}\) uptake may reflect the antiproliferative effect of gefitinib in a mouse model of human epidermoid cancer.”

3) The authors stated that this study was performed to assess FLT as a surrogate biomarker. However, surrogate marker can be studied only when the clinical efficacy was assessed in relation to the biomarker. Again, I would like to stress that this is an animal study to assess early changes after gefitinib.

Response:
We completely agree with the reviewer. In accordance with reviewer’s comment, we have revised the sentences with the expression “\(^{3}\text{H-FLT}\) as a surrogate biomarker” and added the sentence with the expression “in human xenografts” in the abstract, introduction, and discussion in the revised manuscript as follows:

Corrections:
(1) Page 4, lines 13 to 15 in the conclusion of the “Abstract” section:
From: “Thus, it was indicated that \(^{3}\text{H-FLT}\) as a surrogate biomarker can be used for monitoring the antiproliferative effect of gefitinib in a mouse model of a human epidermoid cancer.”
To: Thus, it was indicated that early changes in \(^{3}\text{H-FLT}\) uptake may reflect the antiproliferative effect of gefitinib in a mouse model of a human epidermoid cancer.”

(2) Page 8, line 15 to page 9, 1 in the “Background” section:
From: “Although, several studies have indicated the usefulness of \(^{18}\text{F-FLT}\) or \(^{3}\text{H-FLT}\)
for monitoring effect of gefitinib [25,26], whether $^3$H-FLT as a surrogate biomarker can be used for accurately monitoring the effect of gefitinib by comparing the level of $^3$H-FLT uptake with those of other proliferation or predictive markers, such as Ki-67 or phosphorylated EGFR, in an early phase of treatment has not been fully validated under a pathological condition.”

To: “Although several studies have indicated the ability of $^{18}$F-FLT or $^3$H-FLT to detect the effect of gefitinib [25,26], whether changes in $^{18}$F-FLT uptake can reflect the effect of gefitinib by comparing the level of $^{18}$F-FLT uptake with those of other proliferation or predictive markers, such as Ki-67 or phosphorylated EGFR, in an early phase of treatment has not been fully validated under a pathological condition.”

(3) Page 9, lines 2 to 6 in the “Background” section:

From: “Thus, in the present study, to evaluate whether $^3$H-FLT as a surrogate biomarker can be used for the early and accurate detection of the antiproliferative effect of gefitinib, we determined the changes in $^3$HFLT uptake level after the start of treatment at different doses of gefitinib in comparison with those in $^{18}$F-FDG uptake, Ki-67 expression, and phospho-EGFR levels in a human tumor xenograft (EGFR-dependent human tumor xenograft model, A431).”

To: “Thus, in the present study, to determine whether early changes in $^3$H-FLT uptake can reflect the antiproliferative effect of gefitinib, we determined the changes in $^3$HFLT uptake level after the start of treatment at different doses of gefitinib in comparison with those in $^{18}$F-FDG uptake, Ki-67 expression, and phospho-EGFR levels in a human tumor xenograft (EGFR-dependent human tumor xenograft model, A431).”

(4) Page 22, lines 16 to 18 in the “Discussion” section:

From: “Thus, it was indicated that $^3$H-FLT as a surrogate biomarker can be used for monitoring the antiproliferative effect of gefitinib was indicated in a mouse model of human epidermoid cancer.”

To: “Thus, it was indicated that early changes in $^3$H-FLT uptake may reflect the antiproliferative effect of gefitinib in a mouse model of human epidermoid cancer.”

**Added sentences:**

(1) Page 18, lines 7 to 9 in the “Discussion” section:

“Thus, the measurement of tumor proliferative activity using $^3$H-FLT may enable early accurate assessment of the response to therapy with a molecular targeted drug, gefitinib, in human tumor xenograft.”
(2) Page 19, lines 8 to 9 in the “Discussion” section:

“Thus, our findings suggest that $^3$H-FLT can reflect EGFR activation and can be a predictor of the tumor response to gefitinib in human tumor xenograft.”

We again thank the reviewers for their insightful comments and useful suggestions that have helped us improve our manuscript.