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Reviewer A

Comment 1: some minor language improvements, e.g. in Suppl. Table S1: it should be "forward" instead of "formard"; in the legend of Suppl. figure S1 "Growth of transfected cells..." instead of "Cell growth of transfected cells..."

Reply 1: Thank you for your review and for pointing out the typographical errors. The errors were corrected as suggested by the reviewer.

Changes in the text:

We have revised the misspells in Supplementary Table1, “Formard” to “Forward”.

We have revised the title of Supplementary Figure 1 (revised version of Supplementary Figure 2).

Original	Revised
Supplementary Figure 1	Supplementary Figure 2
Cell growth of transfected Ba/F3 cells with HER2 point mutations.	**Growth of transfected Ba/F3 cells with HER2 point mutations.**

Comment 2: - "HER2" referring to the gene is not handled uniformly (e.g. plain text in lines 37+40+43+43+45+47+54 of the Abstract, vs. italics in lines 44+51+55 of the Abstract). This should be made uniform.

- similarly, in line 355: "we identified a secondary C805S HER2 mutation as well as increased HER3 expression" -> HER3 would be more appropriate in plain text here (instead of the italics used), because it refers to the protein, which is how the authors handled the same issue in line 55 of the Abstract (HER3 in plain text here)

Reply 2: Thank you. We carefully checked the entire manuscript and revised styles for genes (italic) and proteins (plain text).

Changes in the text: There are too many points for which we changed the styles (italic / plain text) for “HER2” or “HER3”. These points are highlighted in yellow in the revised manuscript.

Page 4, lines 48, 51, 54, 54, 56, 58, 60
Page 5, lines 64, 67, 67, 71
Page 10, line 141
Page 13, line 188
Page 17, lines 250, 254
Page 18, line 267
Page 21, lines 317, 319
Page 25, line 381
Page 35, lines 521, 522, 522, 525, 526, 527, 527, 528
Page 36, lines 537, 538, 539, 539, 545, 547
Page 37, lines 549, 549, 551
Page 38, line 579

Comment 3: - explanation of abbreviations in Figure legends (e.g. "TR" in Figure 5)

Reply 3: Explanations of abbreviations were added in each figure legend, including "TR" in Figure 5.

Changes in the text:

The abbreviations were added to Fig. 1 (Page 35, line 524), Fig. 2 (Page 36, lines 541-543), Fig. 3 (Page 37, lines 558-560), Fig. 4 (Page 38, lines 573-574) and Fig. 5 (Page 39, 585-586)

Comment 4: - HER2-directed ADC (e.g. trastuzumab deruxtecan) are also an important strategy against HER2-mutated tumors including NSCLC, this could also be mentioned in the introduction, when the authors give an overview of therapeutic options (while I later saw that the authors have added some data about trastuzumab deruxtecan in the Discussion)

Reply 4: Following the suggestion by the reviewer, we added a sentence describing the trastuzumab deruxtecan (T-DXd) in the Introduction.

Changes in the text:

We added the description about trastuzumab deruxtecan to Background (Pages 7-8, lines 103-106)

"however, 67% (8/12) of patients who had received poziotinib experienced dose reduction due to adverse events, and eight patients (67%) had grade 3-4 AEs (8). Therefore, for HER2-mutated lung cancers, a variety of new therapeutic strategies are currently being tested in clinical trials, including a new drug delivery system (taloxotinib) and an antibody-drug conjugates (trastuzumab deruxtecan (T-DXd) (9)."

Reviewer B

Comment 1: For the experiments that identified 50 clones with HER2 secondary mutations, the authors noted 30 clones with C805S mutation; what are the mutations in the other 20 clones? This information may be useful to the community and should be provided.

Reply 1: Thank you for your review and for your comments. We sequenced tyrosine kinase domain of HER2 to detect secondary mutation, however, no secondary mutation was found in these 20 clones. Therefore, the resistance mechanisms of these 20 clones are unclear. Such phenomenon is sometimes experienced in Ba/F3 models.

Changes in the text: The above information is written in the Results (Page 20, line 295 in the revised version).

"No secondary mutation was found in the HER2 TKD of the remaining 20 clones."

Comment 2: In their investigations of acquired resistance mechanisms of tarloxotinib-E, the authors should note the limitations of the in vitro system: they cannot interrogate adaptive changes in other signaling pathways or assess the possibility of histologic transformation.

Reply 2: The limitations, as suggested by the reviewer, were added in the Discussion.

Changes in the text:

We added descriptions about study limitations as advised by the reviewer (see "Pages 24-25, lines 371-378").

"In this study, we explored acquired resistance mechanisms mediated by secondary HER2 mutations using Ba/F3 models and those mediated by bypass signaling using H1781 cells. However, it is not clear which mechanism, secondary mutations vs. bypass pathway, is more

likely to occur in the actual treatment of HER2-mutated lung cancer patients. In addition, it is possible that other types of resistances, such as histological transformation or modification of tumor microenvironment, may arise. Therefore, future analyses using clinical specimens obtained from tarloxotinib-E refractory patients are necessary to confirm our in vitro findings."

Reviewer C

Comment 1: Fig 2A, Fig 3C, Fig 4A. all Tarloxotinib and Tarloxotinib-E need to show standard derivation, since other competitors indeed showed standard derivation.
Reply 1: Thank you for your review and for your comments. Actually, all the data had standard deviation in our growth inhibitory curves, however, some are shorter than the size of the symbol.
Changes in the text: No change in the text.

Comment 2: If possible, the authors need to show in vivo study in vivid efficacy of Tarloxotinib and Tarloxotinib-E compared to pyrotinib or poziotinibin H1781 xenograft animal model.
Reply 2: Unfortunately, in vivo efficacy could not be texted in this study, however, some data have been presented in the previous study (Estrada-Bernal, et al. Clin Cancer Res 2021).
Changes in the text: No change in the text.

Comment 3: It needs to show chemical structures of Tarloxotinib and Tarloxotinib-E
Reply 3: The chemical structures were added in the Supplementary Figure S1.
Changes in the text: We added Supplementary Figure 1 describing the chemical structures of tarloxotinib and tarloxotinib-E. Due to this change, the numbering of other supplementary figures were changed.