Peer Review File

Article information: https://dx.doi.org/10.21037/tlcr-23-804

Reviewer A

1. The article is good but needs language improvements throughout.
→ In response to your comment, we asked for language correction by a professional medical science editor. We have attached a certificate of editing.

2. The methods could be a bit clearer with more details.
→ We have added additional details to the methods section (page 6, lines 17–18 and page 8, line 9 – 10).

3. The discussion should include more recent articles and also clearly state the limitations of the study.
→ We have added additional recent references (references 30, 32, 34, 36, and 37) and added additional text describing the limitations of this study (page 16, lines 3 –14).

Reviewer B

1. Could the authors elaborate on the varying activity levels of ALK gene fusions encoding chimeric oncoproteins? It might help clarify ALK variants that exhibit a higher dependency on HER3 overexpression.
→ This is an important point. We conducted a comparison of the expression levels of c-MET, EGFR, HER2, and HER3 between the *EML4-ALK* variants V1/V2 and V3a/b in both primary and secondary tissues. There were no significant differences in expression levels among these variants (Table 2). We previously reported that the response differences of the *ALK* fused oncoprotein to ALK-TKIs is largely dependent on the stability of the oncoprotein itself (Woo et al, Ann Oncol. 2017 Apr 1;28(4):791-797. doi: 10.1093/annonc/mdw693). Based on that paper, the unstable V1/V2 subtype of *EML4-ALK* is weaker than the V3 subtype in terms of oncogenicity. Therefore, it is plausible to expect that the instability and low kinase activities of *EML4-ALK* V1 and V2 fusion proteins confer higher dependency on HER3 to drive downstream signalling compared to *EML4-ALK* V3. Thus, the oncogenic effects of the second oncoprotein, HER3, are synergistically escalated in HER3-overexpressing/*EML4-ALK* V1/V2 lung cancers, resulting in poor outcomes. We have added text explaining this more clearly on page 12, line 20 – 23 (result), and discussed on page 15 line 3-8.

2. Additionally, could the authors expand on the role of heregulin (neuroregulin1)? Is there a potential correlation between plasma levels of heregulin and the mechanism of resistance via HER3 upregulation? There is a seminal study demonstrating NRG1's ability to confer resistance to ALK inhibitors in NCI-H3122 cells (as reviewed in Hedge et al., "Blocking NRG1 and other ligand-mediated Her4 signaling enhances the magnitude and duration of the chemotherapeutic response in non-small cell lung cancer," Science Translational Medicine, 2013).

→ Building upon the seminal study you referenced, several *in vitro* studies have shown that upon ligand binding, heregulin1 (HRG1 or NRG1) triggers activation of the HER2/HER3 downstream signaling pathway, implying a role for HER2/HER3 activation in resistance to ALK inhibitors in NSCLC cell lines. We added these findings, along with additional references and the existing reference, to the discussion section (page 14, lines 11–15; references 5, 28, 29).

As you suggested, investigating the functional role of plasma NRG1 expression in HER3-overexpressing lung cancer could be a very interesting topic. A few recent studies have shown that an elevated plasma NRG1 level is significantly associated with EGFR-TKI resistance in patients with NSCLC (Yonesaka *et al*., 2019, Sci Rep) and colorectal cancer (Yonesaka *et al*., 2015, PLos One), as well as trastuzumab resistance in gastric cancer (Sukawa *et al*., 2018, J Clin Oncol). Based on these prior studies, an elevated plasma level of NRG1 may be linked to TKI resistance. We could plan a prospective study with ALK screening to test this hypothesis.

3. What are the thoughts on co-inhibition strategies involving NRG1 and/or HER3/4 antibodies like zenocutuzumab or others?

→ This is an interesting point. With low kinase and intrinsic enzymatic activity, HER3 acts as a dependent heterodimerization partner, relying on phosphorylation from other ERBB members. Zenocutuzumab is considered the most promising bispecific agent for patients with *NRG1* fusion-positive malignancies, including NSCLCs. Additionally, patritumab deruxtecan (HER3-DXd), a HER3-directed antibody-drug conjugate, has shown promising results. Your comments provide new insight into the roles of heregulin1 expression, not solely focusing on HER3 overexpression. Thank you for your comment, and we briefly discussed this along with relevant references in the discussion section (page 15, line 16 – 21; references 36, 37).