Peer Review File

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Reviewer A.

1. One technical question: can the authors provide the IC50 range for the activity of the TKIs listed in Figure 2B? As far as I know, mobocertinib is quite active against EX19 Del+T790M mutant and L858R+T790M mutant; but both were labeled as insensitive.

Reply 1. We can definitely clarify this issue to the reviewer. Our prior preclinical work (reference [81]) disclosed that in common/classical EGFR mutants (exon 19 deletions/indels or L858R) that the presence of EGFR-T790M shifts the IC50 of mobocertinib significantly—as an example: for EGFR- delL747_P753insS (del19) the mobocertinib IC_{50} was 3.97 but for EGFR-delL747_P753insS+T790M was 170 nM (42 times higher); and for EGFR-L858R the IC_{50} was 19.56 but for EGFR-L858R+T790M was 179.10 nM (9 times higher). Our unpublished preclinical data supports the same type of pattern for EGFR exon 20 insertion mutations.

Changes in the text: We will add the following sentence to Figure Legend 2: "B: Summary of preclinical models driven by selected EGFR mutations paired with the in vitro sensitivity and also in vitro selectivity pattern against EGFR wild-type (WT) of the diversity of approved EGFR tyrosine kinase inhibitors (TKIs). Data was extrapolated from references [66, 79 to 82, 84 to 86], and unpublished data from the authors' translational thoracic oncology laboratory. Please, refer to aforementioned references for each individual half maximal inhibitory concentration (IC^{50}) for preclinical proliferation assays.".

2. One edit: references 7-9 were not cited in the body text.

Reply 2. We have corrected this omission.

Changes in the text 2. The following sentence now reads: "Both larger entities can then be further subdivided into clinically relevant subgroups based on tumor biomarkers [7–12].".

3. The authors should read through the manuscript one more time to fix a few obvious typos.

Reply 3. This was reviewed.

Changes in the text 3. Minor typos were fixed. We also modified throughout the abstract and text the new name for CLN-081: zipalertinib.

Reviewer B.

No queries to address.

Reviewer C.

1. Figure 1A – can the percent values be put into a supplementary section table to be more informative for the reader

Reply 1. We appreciate this recommendation. To keep the clean aesthetic of the figure, and to avoid the addition of a supplementary table, we incorporated the expected ranges of genomic alterations in the figure legend.

Changes in the text 1. We will add the following sentence to the legend of Figure 1: "… histologies (data adapted from references 7 to 16: for adenocarcinoma the prevalence of *EGFR* mutations is approximately 15-40%, *ALK* rearrangements 3-5%, *MET* exon 14 skipping mutations 2-4%, *BRAF*-V600E mutation 1-2%, *ERBB2/HER2* mutations 1-2%, *ROS1* rearrangements 1%, *RET* rearrangements 1%, *NTRK* rearrangements 0.1%, and *KRAS*-G12C mutation 15-35%; for squamous cell carcinoma the prevalence of *KRAS-G12C* is approximately 2-10%, *EGFR* mutations 1-5%, and the rare targets of *BRAF*-V600E mutations, *MET* exon 14 skipping mutations, *ALK* rearrangements, *ROS1* rearrangements, *RET* rearrangements, *ERBB2/HER2* mutations, and *NTRK* rearrangements: all less than 0.5%).

2. Figure 2A – please add a note about the numbering of the exons above the diagram for the EGFR gene exons. Is there a similar profiling for HER2 TKIs?

Reply 2. We appreciate this recommendation.

Changes in the text 2. We have modified the legend of Figure 2: "A: Representation of the EGFR protein by key gene numbers, overlaid with clinically-relevant types of mutations mostly centered within the kinase domain. The prevalence of these mutation subtypes are indicated by exon location.".

3. Figure 2B – please explain the significance of +, ++, +++, -, --, ---.

Reply 3. We appreciate this recommendation.

Changes in the text 3. We have added to the legend of Figure 2: "The degree of sensitivity and resistance is indicated by number of + (sensitive/selective) or – (resistant/non-selective) signs as

extrapolated from preclinical studies. Please, refer to aforementioned references for each individual half maximal inhibitory concentration (IC_{50}) for preclinical proliferation assays.".

4. In the introduction, please review the synonyms of the genes encoding and the proteins covered by EGFR and erbB2.

Reply 4. This was already provided in simplified form in the following sentence: "ErbB-1 (also known as epidermal growth factor receptor, EGFR) and ErbB-2 (also known as Her2)". See Line 109.

5. Line 104-105 – is there any dominance of the oncogenic driver mutations that are mentioned in lung cancers?

Reply 5. This was provided in Figure 1 and prevalence of actionable driver oncogenes added to the legend of Figure 1.

6. Line 151 – why are there 1st, 2nd, 3rd generation TKIs? Consider stating whether the mutations occur more in the cancer cell types but not in normal tissues

Reply 6. The nomenclature of 1st, 2nd and 3rd generation EGFR inhibitors is based on the date/ phase of clinical development of different EGFR inhibitors for the most common EGFR mutants. Figure 2 describes the FDA-approved drugs that fit this conventional nomenclature.

7. Line 205 – are there other ADME considerations that can differentiate the different EGFR TKIs? Please consider making commentary on the lung cancers that are autocrine, paracrine, or juxtacrine EGFR engagers

Reply 7. Great question. To the authors' knowledge the main differences in activity and frequency of adverse events to different EGFR inhibitors is due to "therapeutic windows" between WT-EGFR and mutant EGFR (as explained in Figure 2B) instead of ADME-PK/PD differences. In view of this observation, we elect to refrain from adding additional information on ADME parameters for this shared class of EGFR tyrosine kinase inhibitors.

8. Line 233 – comment that the antibodies bind regions of EGFR that are invariant in the mutant classes that are highlighted in Figures 1, 2.

Reply 8. Important observation.

Changes in the text 8. We have added the following sentence to the introduction: "Amivantamab (formerly called JNJ-61186372) is a bispecific antibody targeting extracellular domain of EGFR-MET that shows preclinical activity in TKI-sensitive and TKI-resistant *EGFR*-mutated NSCLC models.".

9. Line 300 – any commentary on dose limiting toxicities that constrain EGFR or HER2 therapies?

Reply 9. Toxicities are clinically relevant for use of newly approved EGFR and ERBB2 targeted therapies. We have highlighted for each approved drug (mobocertinib, amivantamab and T-DXd) major toxicities and their relevance.

10. Line 394 – please add the mechanism for trogocytosis as note in Viyaraghavan et al., (2020) Mol Cancer Ther 19(10) 2044

Reply 10. Thanks for this suggestion.

Changes in the text 10. We have added the modification to the following sentence explaining amivantamab: "Three distinct mechanisms inhibit tumor growth in the setting of aberrant EGFR and MET signaling: 1) immune effector cells (such as NK cells and macrophages) target mutated tumor cells for destruction through antibody-dependent cellular cytotoxicity, trogocytosis and phagocytosis [89]; 2) inhibition of ligand binding prevents ligand-induced activation, phosphorylation, and downstream signaling that promote cellular proliferation; and 3) downmodulation or degradation of EGFR and MET receptors, thereby decreasing tumor volume [89]."

11. Line 429 – any commentary of the dosing levels of lazertinib in combination of amivantamamab in CHRSYALIS and carboplatin and pemetrexed in combination with amivantamamab? Do the combination dosing match what was prescribed in TKI therapy alone?

Reply 11. To the best of the authors' knowledge, the full doses of each individual drug are used for the aforementioned combinatory clinical trials.