Peer Review File

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Review Comments-Round 1

Reviewer A

Thank you for your interesting manuscript, providing an informative and critical insight into new potential druggable alterations. Please find the detailed comments below.

Filetti et al. have prepared a narrative review of new driver mutations in NSCLC.
It is a good, well-written and concise review providing an insight into the current data of new potential genomic alterations in NSCLC. Particularly the tables at the end of each capital enable the readers to get a quick and highly informative approach. Moreover, I am really impressed of the discussion capital being very consistent and giving critical insight into different aspects of the era of personalized medicine. As the authors rightly conclude in the discussion summarizing the description of the KRAS, PIK3CA, NRG1, HER2 and FGFR genes, the state of the current, very quickly progressing research on new alternations shows that there is a constant gap between the results of preclinical studies, and a proper understanding of the role of individual molecular abnormalities, including need of more replication models providing solid evidence for what the future steps of targeted treatment should look like.
However, there are some minor inaccuracies to be explained and a few questions:

Abstract

– please specify which alterations you are going to discuss in the paper: KRAS, PIK3CA, NRG1, HER2, FGFR. You can also provide the reason why have you chosen these genes and why the review should contribute to the current knowledge: Beyond the fact that they are new, it is important to mention that some of these recently become no longer undruggable.
Furthermore, please pay attention that the title can be misleading, since some drivers

you mention are not mutation only, like NRG1-fusion or FGFR may work as oncodriver as amplified, mutated of rearranged. So, the title will be more correct while using “new driver alterations” instead of “new driver mutations”.

Reply 1: Thanks for the suggestions. We have modified and integrated suggestions in the abstract.
Changes in the text: See page 2 line 20-41

Introduction
- Is the copy of the abstract and does not provide more information.
The role of abstract and introduction are different. Please consider rephrasing them, so the abstract shows the essence of the problem you discuss, and the introduction outlines a little wider the current status and informs about the potential challenges with these new alterations.

Reply 2: Thanks for the suggestion. We have included a revised version of the introduction.
Changes in the text 2: See page 3 line 46-71

KRAS
- please extend the abbreviations: KL, KP and KC while characterizing the three subgroups of KRAS coexisting mutations.
- line 120 (IMA) – extend the abbreviation ok
- line 128: the KRAS G12C mutation accounts for 8-13% of all pulmonary adenocarcinomas. Otherwise explain where does the 41% come from, thank you.

Reply 3: Thanks for the suggestion. We corrected inaccuracies and errors.

- line 138: Consider extending the mechanism of inhibiting this mutation. KRAS-G12C can circulate actively between the GDP and GTP-related states, maintaining interaction with its downstream effectors. This difference allows the protein to be specifically inhibited by blocking it in its inactive conformation by targeting the reactive cysteine residue 38 by new KRAS G12C inhibitors binding to

GDP causing inactive state. Therefore, it is important to underline that inhibiting of KRAS G12C is a unique mechanism, where Sotorasib captures the G12C in GDP-mode. This is in contrary to other known mechanisms of TKI-acting.

Reply 4: Thanks for the suggestion. We added a more comprehensive explanation of the mechanism of action.
Changes in the text: see line 114-120

- either extend all the names of genes and abbreviations or not. If you do, so please extend also KRAS. Ok fatto
- line 157: Table 1. Please provide the references to the citated studies, as you did in table 3 and 4. However, please choose the same way, either by making additional column like in table 3 or mark the numbers for references like in table 4. It should match sequence of the references you narrate in the text.

Reply 5: Thanks for the suggestion. We corrected inaccuracies and errors.

PIK3CA
- line 232: table 2. Please provide references (as mentioned for table 1)
-line 223/230: it is a particularly good refection

Reply 6: Thanks for the suggestion. We added references for Table 2.

NRG1
- line 263: please add to be clear for readers that NRG1 proteins generally not expressed in normal lung (like e.g., ALK proteins). Therefore, NRG1 overexpression is a priori supposed to be pathogenic.

- line 265: this is physiological binding, because NRG1-protein serves as a ligand for ERBB2 and ERBB3 and causes ERBB2 and ERBB3 heterodimerization. Whereas NRG1-fusioned protein, preserving EGF-like domain, stimulates continuously this PI3K/AKT pathway.
- line 271: please provide reference to the only one squamous cell carcinoma of the

lung with SMAD4-NRG1 Fusion.

- 278; coma lacks between “histotypes” and “NRG1”

HER2

- line 332: coma is lacking after “Buttitta et al”
- line 333: please explain: “concurrent ERBB2 gene amplification” – concurrent to what? Do you mean coexisting mutation and amplification of ERBB2?

It important to underline that HER2 can appear in three abnormal independent states: mutation, amplification, and protein overexpression.

- line 350: while the most frequent (25,5%) in EGFR exon 20 is mutation isD770-N771insX (Remon J, Cancer Treat Review, 2020)

- line 341: Exon 20 alterations of EGFR and HER2 have similar crystal structures and biological functions, including the α-C helix (residues 762–766 in EGFR and 770–774 in HER2) and the loop following the α-C helix (residues 767–774 in EGFR and 775–783 in HER2). You wrote that “Their exon 20 consists of two regions the α-C helix and the loop following the α-C helix”. Please prove that exon 20 consists of two regions the α-C helix? Otherwise rephrase the sentence that it is one α-C helix.

- line 357: However, EGFR ex20ins A763_Y764insFQEA – unique variant sensitive to first- or second-generation EGFR-TKI (Remon J, Cancer Treat Review, 2020).

- line 394: remove “(“
- line 414: please specify/add: “...and fluorescent in situ hybridization (FISH) for amplification and next-generation sequencing (NGS) for mutations and amplifications”.
- line 418: specify in the table legend: “UN” and “N/A” mentioned for number of patients in Pyrotinib study

FGFR

- line:426 alterations occur “as” not “by”

- line 478: table 5: add “n.a.” to the legend or change to N/A as mentioned in table 4 to make them unified.

Reply 7: Thanks for the suggestions. We corrected the mistakes.

Discussion

- line 488: I would discuss that it is not so bad for some other targets as e.g., ALK-positive NSCLC where median OS on ALK-TKIs is over 7 years. (Duruisseaux et al. Oncotarget 2017;8;21903-21917, Pacheco JM. et al. Natural History and Factors Associated with Overall Survival in Stage IV ALK-Rearranged Non–Small Cell Lung Cancer. J Thorac Oncol. 2019; 14(4): 691–700. doi: 10.1016/j.jtho.2018. 12.014), which is currently the best OS value for metastatic genomic defined NSCLC. However, I will agree that targeting the new alterations mentioned in the paper, like PIK3CA and FGFR are still incredibly challenging. I agree that the problem is to understand what the results of preclinical studies really mean for the development of therapies for individual targets.

Reply 8: Thanks for the suggestions. We wrote a new version of the discussion following your suggestions

Changes in the text: see lines 450-470

Reviewer B

Although the subject of this manuscript (MS) is relevant and interesting, several and more systematically written reviews have recently been published on new targets in lung cancer. Therefore, the MS suffers from its relative lack of novelty and from its presentation that appears confused and rushed-trough without proper check of its structure. A major revision of the English form and the formatting of the MS as well as the addition of apparently missing parts is necessary before it can be publishable. Despite claiming that the MS is based on an extensive review of the literature, including published articles and congress abstracts from the last 5 years, some recent relevant reports in the field are not mentioned. There are several studies named in text with the wrong reference or even without corresponding references, which per definition are essential in a review article.

FURTHER SPECIFIC POINTS

The title should reflect more properly the purpose indicated by the authors for their review, i.e. to present "the current state of the art on new molecular drivers in NSCLC with a focus on new drugs in early development". The title should be modified accordingly. Also, it is unclear what the authors mean with "narrative review". Is this specification necessary, especially when the purely descriptive aspect of the review is not optimal?

Abstract and Introduction are identical. The Introduction session should introduce more extensively the unmet therapeutic needs of the last decade or so and the issue of novel emerging (and possibly other potential) targets in NSCLC.

Abstract and Introduction, sentence “Recently other mutations, such as those affecting BRAF, and RET or NTRK rearrangements have revealed very interesting data, obtaining accelerated approvals from the FDA and EMA.” It should be rephrased, as it sounds like the mutations are revealing data (like persons) and have been approved. Drugs targeting these mutations have been approved, not the mutations themselves …

The authors have written the review considering “all potential drivers with ongoing phase II or III studies, for which there are currently no FDA or EMA approvals”. Again, this should be reformulated, as drugs against these drivers, not the drivers themselves, are approved.

In addition, the FDA granted in May 2021 accelerated approval to Sotorasib for KRAS G12C-mutated NSCLC, thus the inclusion criteria for the review are debatable. In this respect, it is not completely clear then, why BRAF, RET, and NTRK1-3 (and MET) are not included as novel targets.

Reply 1: Thanks for the valuable comments. We have proceeded to restructure the abstract and introduction adequately.

Changes in the text: See page 2-3, line 20-71

When describing each target, the authors should be more systematic and consistent. First a brief description of the biology related to the target and then an account of drugs against that target and related clinical trials. As the MS is written now, for some targets the biological notions are mentioned after presenting the clinical studies.

Line 95-96, “KRAS-mutation … is more widely represented in adenocarcinoma, with a prevalence of 20-40% versus 5% of squamous cell carcinoma”. It would be worth specifying that KRAS-mutations are detectable at a frequency of 20–40% in Caucasian patients and 2–10% in Asian patients, in contrast to the opposite frequency trend of EGFR-mutations in the two populations.
Line 127-8, “The most common mutation is the KRAS G12C (41%) followed by KRAS G12V (7%) and KRAS G12D”: Some reference is necessary here to support these data.

Reply 2: Thanks for the suggestions. We corrected mistakes and added the correct reference.

Line 135-6, “have shown early promise results”: presumably it should be “have shown early promising results”.

Reply 3: Thanks for the suggestion. We corrected the mistake.

Line 141-3, “Phase II data were recently submitted to the 2021 IASLC World Conference; 126 patients with KRASG12C mutated NSCLC were included [8]”: The authors may want to refer to the more recent and updated data of the Phase II trial just published in NEJM: Skoulidis F et al. Sotorasib for Lung Cancers with KRAS p.G12C Mutation. N Engl J Med 2021; 384:2371-2381. DOI: 10.1056/NEJMoa2103695

Reply 4: Thanks for the suggestion. We added the correct reference

Line 148, “also shown activity in”: also showed activity in

Reply 5: Thanks for the suggestion. We corrected the mistake.

Line 153-5, “These studies highlighted the complexity of targeting KRAS-mutated cancer. The difficulty arises mainly from the presence of many different aberrations and co-mutations that probably modulate tumor biology and response to therapy”: In

this respect, the authors should cite and discuss the recent article by Awad MM et al. Acquired Resistance to KRASG12C Inhibition in Cancer. N Engl J Med 2021; 384:2382-2393. DOI: 10.1056/NEJMoa2105281, that illustrates the genetic and phenotypical mechanisms of acquired resistance identified in patients treated with Adagrasib monotherapy.

Reply 6: Thanks for the suggestion. We added discussion of the work in the paragraph.
Change in the text: see lines 136-144

Tables 1, 2, 4, and 5 need corresponding references for each single result to be meaningful!

Reply 7: Thanks for the suggestion. We added the corresponding references for each table.

Table 1, 2nd line, Sotorasib phase II trial: In the NEJM Phase II article mentioned above (Skoulidis et al. DOI: 10.1056/NEJMoa2103695), ORR was still 37%, while DCR was 81%, median PFS 6.8 months, duration of response 11.1 months) and mOS 12.5 months.

Reply 8: Thanks for the suggestion. We corrected the mistake.

Line 187-90, "This properly reflects what we observed in clinical practice, … [21]": the sentence is unclear, as none of the authors is among the authors of reference 21. It should be rephrased more neutrally (for ex. "what can be observed in clinical practice …").

Reply 9: Thanks for the suggestion. We corrected the mistake.

Line 190-1, "To date, available data on early phase clinical trials did not provide satisfying results": To date, available data on early phase clinical trials have not provided satisfying results.

Reply 10: Thanks for the suggestion. We corrected the mistake.

Line 197, “had likewise fail to” should be “had likewise failed to”.

Reply 11: Thanks for the suggestion. We corrected the mistake.

Line 207, “in combination with Erlotinib in patients previously progressed”: in combination with Erlotinib in patients who previously progressed (or previously progressing).

Reply 12: Thanks for the suggestion. We corrected the mistake.

Line 221-3, “Notably, none of these agents received approval for the treatment of NSCLC yet, and the lack of efficacy markedly slowed down”: Notably, none of these agents have received approval for the treatment of NSCLC yet, and the lack of efficacy has markedly slowed down.

Reply 13: Thanks for the suggestion. We corrected the mistake.

Line 229, “Thus, a PI3K mutation should not be considered as a driver mutation”: this is a strong statement. The current data do not exclude that PI3K-mutations are co-drivers, at least in the context of acquired (and possibly primary) resistance to TKIs. If resistant, PI3K-mutated clones emerge, the mutations must have some driving effect.

Reply 14: Thanks for the suggestion. We reworded the paragraph.

Line 260, “the homonym gene”: homonym is a linguistic term referred to words with different meanings but same spelling and pronunciation. The term “homonymous gene” would be more appropriate in a biological/medical context.

Reply 15: Thanks for the suggestion. We corrected the mistake.

Line 266-7, “Rearrangements involving Neuregulin-1 (NRG1) gene are described in lung adenocarcinoma (LUAC)”: especially in the mucinous subtype.

Reply 16: Thanks for the suggestion. We corrected the mistake.

Line 271, “reported in the literature harbor SMAD4-NRG1”: reported in the literature harbored SMAD4-NRG1.

Reply 17: Thanks for the suggestion. We corrected the mistake.

Line 277-8, “immunohistochemistry for pErbB3 has been proposed as screening test to suspect NRG1 fusion”: some reference should be provided to support this statement. For ex.:
Trombetta D et al Oncotarget. 2018 Feb 9; 9(11): 9661–71 and/or Laskin J et al. Ann Oncol. DOI:https://doi.org/10.1016/j.annonc.2020.08.2335.

Reply 18: Thanks for the suggestion. We added the reference.

Table 3, line 1 Afatinib, N. of patients 11: It should be 12 according to what described also on line 292.

Reply 19: Thanks for the suggestion. We corrected the mistake.

In the same table 3 all references are wrong!: According to the reference list Duruisseaux M et al. [28] should be 58); Drilon A et al. [17] should be 54; Gay ND et al. [29] should be 59; Han JY et al. [30] should be 60; Schram AM et al. [31] should be 61.

Reply 20: Thanks for the suggestion. We corrected the mistake.

The authors mainly focus on HER2 ex20ins but ERBB2-mutations can affect also the extracellular (exon 5-8) and the transmembrane (exon 17) domains, though they are

much more frequent in the TKD (exon 18-24). Similar to EGFR-mutations, they can result in substitutions, ex19dels, and in-frame ex20ins or duplications, which are the most frequent ERBB2-mutants in pulmonary adenocarcinoma. A sort of introductive sentence like this, would have been appropriate in the beginning of the section on HER2.

Reply 21: Thanks for the suggestion. Implemented the introduction with the suggested information.
Changes in the text: see line 301-314

Line 327-9, the sentence "The mutation outnumbers in women, with a median age at diagnosis of 60 years, in never smokers, and in Asian population when compared with other ethnicities [65]" should be rephrased in more proper English.
Line 329-30, "HER2 mutated LUACs are moderate to poorly differentiated with high grade morphology": Unclear what the authors mean by that, it sounds as a repetition (poorly differentiated = high grade morphology). In any case, in reference 66, table 3, Li C et al. describe the "Clinical and Molecular Characteristics of the Eight Samples with HER2 Mutation" and show that they were predominantly moderate to poorly differentiated adenocarcinomas. They do not describe the tumor grade, only differentiation. Line 330-3, "In the case series by Arcilia et al. mutated tumors have a mixed phenotype with papillary, micropapillary, acinar and solid predominant pattern, whereas in the Buttitta et al series bronchioloalveolar carcinoma features have been frequently observed": please provide both in the text and reference list the references for the mentioned studies by Arcilia et al. and Buttitta et al. They are both missing …
Line 335-7, "However, only a minority of the samples show significant immunohistochemical overexpression of HER2 protein, indicating that mutation alone does not seem to be associated with increased protein expression": do the authors mean HER2 mutation or amplification? The latter would often result in overexpression of HER2 protein, the former would not. Please specify.
Line 344-6, "When exon 20 insertions occur, the C-helix has a permanent active conformation, with enhanced survival, invasiveness, and tumorigenicity": please specify what is acquiring enhanced survival, invasiveness, and tumorigenicity after occurrence of ex20ins.

Line 366-7, “Neratinib was studied in combination with the mTOR inhibitor in a preclinical setting”: it should be named which mTOR inhibitor, as it has not been mentioned before. According to the cited meeting abstract it was Temsirolimus.

Reply 22: Thanks for the suggestion. We have rewritten the paragraph following your precious indications.

Line 371-2, “but the only published results are for EGFR exon 20 mutations. [76]”: here it could be cited and briefly discussed the newer published results in Gonzalvez F et al. Mobocertinib (TAK-788): A Targeted Inhibitor of EGFR Exon 20 Insertion Mutants in Non–Small Cell Lung Cancer. Cancer Discov 2021;11:1672–87. In this report, the in vitro and in vivo activity of Mobocertinib was studied in engineered and patient-derived models harboring diverse EGFRex20ins mutations showing clear superiority over other approved EGFR-TKIs.

Reply 23: Thanks for the suggestion. We added the suggested paper.

Line 393-4, “T-DM1 presented activity in both exon 20 insertion, point mutations, and HER2 amplification”: the corresponding reference is missing from the text.

Reply 24: Thanks for the suggestion. We corrected the mistake.

Line 398, “was tested in a phase I clinical trial recruited patients”: was tested in a phase I clinical trial that recruited patients.

Reply 25: Thanks for the suggestion. We corrected the mistake.

Line 403-6, “In the RAIN trial patients treated with tarloxotinib obtained an ORR 22% and … (95% CI, 6.4-14.0 mo) [84-85]”: The paragraph and the cited references 84-85 do not have anything to do with Tarloxitinib, but with trastuzumab deruxtecan/DS-8201a. Tarloxotinib and the RAIN trial should be described separately. The authors should explain what Tarloxotinib is. For ex. they could cite and explain Estrada-Bernal A et al. Tarloxotinib Is a Hypoxia-Activated Pan-HER Kinase

Inhibitor Active Against a Broad Range of HER-Family Oncogenes. Clin Cancer Res 2021;27:1463–75, where they show that it is a prodrug activated by tumor hypoxia to generate high levels of a potent, covalent pan-HER tyrosine kinase inhibitor (tarloxotinib-effector) displaying activity in patient-derived cell lines and xenografts harboring EGFR exon 20 insertion mutations, HER2 mutations and amplification, and NRG1 fusions, as well as in pts with HER2 ex20ins.

Moreover, the RAIN trial should be cited properly:
Liu SV et al. LBA61 First analysis of RAIN-701: Study of tarloxotinib in patients with non-small cell lung cancer (NSCLC) EGFR Exon 20 insertion, HER2-activating mutations & other solid tumours with NRG1/ERBB gene fusions. Ann Oncol VOLUME 31, SUPPLEMENT 4, S1189, SEPTEMBER 01, 2020. DOI:https://doi.org/10.1016/j.annonc.2020.08.2294

Reply 26: Thanks for the suggestion. We corrected the mistake and added a paragraph for Tarloxotinib.

Line 412-5, “To present, there are no gold standards, and the laboratory methods used consist of immunohistochemistry (IHC) for overexpression of proteins and fluorescent in situ hybridization (FISH), and next-generation sequencing (NGS) for genetic alterations”: 1) “To present” should be “at present” or “currently”; 2) It is totally unclear what is the point of the sentence. The same methods are used worldwide for HER2 and many other therapeutically relevant genetic alterations in NSCLC. Moreover, a discussion on methodology seems to be beyond the scope of this review.

Reply 27: Thanks for the suggestion. We deleted the paragraph.

For completeness the authors should also mention the occurrence and relevance of HER2 mutations in pulmonary SqCC. For ex., they could cite Hamada A et al. P86.05 In Vitro Validation Study for HER2 Mutations Identified in Secondary Analysis of the LUX-Lung 8 Randomized Clinical Trial. J Thorac Oncol. Vol. 16, ISSUE 3, S673-S674, MARCH 01, 2021. DOI: https://doi.org/10.1016/j.jtho.2021.01.1234. It

shows that in SqCC certain HER2 mutations had transforming activity and 2nd/3rd generation TKIs, including Afatinib, were more effective than Erlotinib, suggesting that HER2 mutations should also be investigated in pulmonary Sqcc pts.

Line 429, “Weiss et al. analysed”: there is no reference for Weiss et al. in the text and reference list!

Reply 28: Thanks for the suggestion. We corrected the mistake.

Line 431-2, “Examination of an independent series by FISH revealed”: again, no reference is provided for this study.

Reply 29: Thanks for the suggestion. We corrected the mistake.

Line 434-6, “Helsten et al. describe FGFR2-3 mutation in 3% of LUSC, and any abnormalities of FGFR in 4% of LUAC [86].”: Helsten et al is not reference 86, in the reference list is nr 90!

Reply 30: Thanks for the suggestion. We corrected the mistake.

Line 465-6, “Pemigatinib is currently testing in various malignancies”: Pemigatinib is currently being tested in various malignancies.

Reply 31: Thanks for the suggestion. We corrected the mistake.

Line 469-72, “The main obstacle for FGFR inhibitors is the extreme variability that cancer cells present for this class of drugs. Preclinical studies showed there is no direct correlation between genetic alteration and sensitivity to the associated target drug, and that not all cells respond equally to inhibitors.”: these sentences are rather cryptic and are not supported by any references. They should be rephrased more clearly and with related reference citations.

Reply 32: Thanks for the suggestion. We rephrased paragraph as suggested.

The Discussion is not appropriate. It is speculative, out of context, and very negative towards current precision medicine in NSCLC, which after all has made enormous progress in the last decade, compared to one-size-fits-all chemotherapy of the past. It does not summarize the descriptive paragraphs on novel targets and their main take-home message. Obvious things, such as differences between driver mutations, passenger mutations, and acquired resistance mutations seem not necessary, as they are very well known to readers in the field. Instead, the problem of tumor heterogeneity/co-mutations and future perspectives related to novel combination targeted therapies could have been highlighted in the Discussion.

Reply 33: Thanks for the suggestion. We wrote a new version of the discussion following your suggestions

Changes in the text: see lines 450-470

The reference list needs definitely attention: 1) Some references lack pages, issue number and year of publication (for ex. reference 2-5, 9, 26, 80, 82, just to mention some I come across). 2) Sometimes, references with several authors are written as names et al., other times as names ... last author's name. Only one system should be used. 3) Is it necessary to cite Epub dates? And also to cite both doi and PMID?

Reply 34: Thanks for the suggestion. We corrected the reference list as suggested

Changes in the text: see lines 480-820

Review Comments-Round 2

Reviewer B

The manuscript has been improved by implementing several of the suggested adjustments. However, there remain structural and conceptual problems. Moreover, the authors have not provided an entirely satisfactory point-by-point reply to the initial comments. Most of their replies to the initial comments are “Thanks for the suggestion. We corrected the mistake”, which is a kind type of reply, but does not

explain how and where the text of the manuscript has been changed (and line numeration differs now from that in the originally submitted version). Thus, it has been difficult to find the actual changes that had been suggested and verify that they are correct. Moreover, some of the modifications that the authors claim to have introduced in the text, are not visible.

SPECIFIC POINTS

(New) line 76-77, "KRAS mutation is one of the most prevalent in NSCLC (3). It is more widely represented in adenocarcinoma, with a prevalence of 20-40%": This applies to Caucasians. It is still missing the fact that KRAS-mutations are detectable at a frequency of 20–40% in Caucasian patients and 2–10% in Asian patients, in contrast to the opposite frequency trend of EGFR-mutations in the two populations.

Reply 1: Thanks for the critical clarification. We have taken steps to integrate the information and add a supporting reference. See line 77-79

Line 123-124, "The drug was presented in 2020 in a Phase I study involving patients with pretreated KRAS mutated solid tumors": Reference 11 (Hong DS et al NEJM 2020) should be cited here. The drug was already "presented" at the IASCLC's WLC in Barcelona 2019, thus it would be more appropriate to state that 2the results of phase I study were published in 2020".

Reply 2: Thank you for the clarification. We rephrased the sentence by specifying the chronology better. See line 124-127

Line 126-127, "Phase II data were recently submitted to the 2021 IASLC World Conference; 126 patients with KRASG12C mutated NSCLC were included (13)": Reference 13 is a meeting abstract (presumably for the 2021 IASLC WCLC), but the source is not cited in the reference list. This also is the case for other cited abstracts (for ex. 84). The abstract book/Proceedings the reference is obtained from should be properly cited for the readers.

Moreover, as already suggested to the authors in the initial review of their manuscript, they should cite here the recently published article on the Phase II trial in NEJM: Skoulidis F et al. Sotorasib for Lung Cancers with KRAS p.G12C Mutation. N Engl J

Med 2021; 384:2371-2381. DOI: 10.1056/NEJMoa2103695 (in case use this citation and not the abstract in 13).

Reply 3: Thanks for the clarification. We replaced the reference no.14.

Table 1: Following problems in the sections on 1st line Sotorasib Phase I trial and 2nd line Phase II trial need to be amended:

- reference 11 is incomplete in the reference list;
- the authors have mixed the results of Phase I and II trials. In phase I trial conducted on pts with different solid tumors (ref. 11), the subgroup with NSCLC showed ORR 32.2% (CR or PR), DCR 88.1% (OR or SD); mPFS was 6.3 months; in the phase II trial (again refer here to Skoulidis F et al. DOI: 10.1056/NEJMoa2103695), ORR was 37%, DCR 81%, mPFS 6.8 months, duration of response 11.1 months and mOS 12.5 months.

Reply 4: Thanks for the clarification. We entered full references and adjusted the data from the two studies. See Table 1

Line 299, Section 5 on *HER-2*: In other parts of the manuscript, the authors have used the gene name *ERBB2*, which corresponds to the newer nomenclature in the *ERBB* gene family. It would be appropriate to be consistent throughout the whole manuscript, i.e. either using *HER2* or *ERBB2*, but not both or if that's the case, the nomenclature should be explained (for clarity).

Reply 5: Thanks for the comment. We uniformed the nomenclature as suggested.

Line 302-304, "In general, alterations of the *HER-2* gene can be divided into three subgroups: mutation, amplification, and protein overexpression": Protein overexpression is not correctly defined as a "gene alteration" (even though, it can be caused by a gene alteration). Furthermore, it has to be clear for the readers that protein overexpression is not always due to alteration of the *HER2* gene. Typically *HER2* gene amplification leads to protein overexpression, but this can also occurr due to transcriptional mechanisms or post-trasncriptional regulation, such as increase protein stability. Thus, some rephrasing seems appropriate in the sentence.

Reply 6: We inserted a sentence to clarify the classification better and specify the causes of the overexpression. See line 304-308

The authors only deal with *HER-2* ex20ins. In contrast to what they claim in the rebuttal to the initial comments (reply 21 to reviewer B), they have not followed the suggestion of introducing the subject of *HER-2* mutations by explaining that they can also occur in the extracellular (exon 5-8) and the transmembrane (exon 17) domains, but they are much more frequent in the tyrosine kinase domain (TKD = exon 18-24) as it also the case for EGFR. Similar to EGFR-mutations, the mutants in the TKD can be substitutions, ex19dels, and in-frame ex20ins or duplications, which are the most frequent *HER-2* mutants in pulmonary adenocarcinoma.

Reply 7: Thanks for the kind clarification; we reworded the introduction by specifying better the various types of mutation. See line 308-312

Line 309-310, "However, only a minority of the samples show significant immunohistochemical overexpression of HER-2 protein": Add "of the samples with *HER-2* mutation" to avoid confusion, as samples with *HER-2* amplification usually show protein overexpression.

Reply 8: Thanks for the suggestion; we edited the sentence as suggested. See line 316-317

Line 318-319, "When exon 20 insertions occur, the C-helix has a permanent active conformation, enhanced survival, invasiveness, and tumorigenicity": rephrase more properly the sentence as for ex.: "When exon 20 insertions occur, the C-helix changes to a permanent active conformation, resulting in enhanced survival, invasiveness, and tumorigenicity of the cells harboring these mutants". Otherwise, it sounds like the C-helix itself has enhanced survival and invasiveness.

Reply 9: Thanks for the suggestion; we edited the sentence as suggested. See line 326-328

Line 322-323, “These alterations do not increase the affinity for EGFR TKIs, because they did not concern the ATP-binding pocket”: it should be “These alterations do not increase the affinity for EGFR TKIs, because they do not concern the ATP-binding pocket”. Furthermore, the part of the sentence “do not concern the ATP-binding pocket” should be explained more completely. It is true that ex20ins, as opposed to activating mutations in other exons, do not change the ATP-binding pocket conformation. However, it should be added that, instead, they activate EGFR or HER-2 by altering the conformation and relieving key autoinhibitory interactions within the C-helix of the TKD. This leads to sterical hindrance of the TKI-binding to the activated receptor. Thus, also in this case it is a matter of sterical interference of the drug-binding.

Reply 10: Thanks for the suggestion; we specified the concept better. See line 331-334

Line 337-338, “In a phase II study, dacomitinib showed a partial response on 3 of 26 patients with HER-2 mutations or amplifications (83)”: There are additional, quite relevant, and newer references on the subject that may be worth citing for the sake of completeness and value of this review article. In particular, publications showing the variable response of NSCLC patients carrying HER-2 or EGFR ex20ins mutants to 2nd generation EGFR-TKIS such afatinib, dacomitinib og neratinib:

Mazières J et al. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeteddrugs: Results from the European EUHER2 cohort. Ann. Oncol. 2016,27, 281–28

Kosaka T et al. Response heterogeneity of EGFR and HER2 exon 20 insertions to covalent EGFR and HER2inhibitors. Cancer Res. 2017,77, 2712–272

Robichaux J.P et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat. Med. 2018,24, 638–64.

Oh I.J.; et al. Clinical activity of Pan-HER inhibitors against HER2-mutant lung

adenocarcinoma. Clin. Lung Cancer 2018,19,e775–e78

Hyman D.M et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 2018,554,189–194.

Reply 11: Thanks for the comment. We revised the period by integrating suggested data. See line 350-358

Line 369-372, "Preliminary data from the DESTINY-Lung01 trials were recently presented and showed ….. PFS of 14.0 mo (95% CI, 6.4-14.0 mo) (94-95)": references 94-95 are from abstracts of scientific meetings (and lack year of publication in the reference list, see comment on reference list underneath). They can be substituted by the just published article (Sept. 18, 2021) on the multicenter DESTINY-Lung01 trial, in which the data also are more mature: Yasushi G et al. Trastuzumab Deruxtecan in *HER2*-Mutant Non–Small-Cell Lung Cancer. NEJM DOI: 10.1056/NEJMoa2112431

Reply 12: Thanks for the suggestion; we proceeded to update the sentence by inserting the most recent reference. See line 391-394

Line 375-376, "In preclinical studies, tarloxotinib exhibited promising activity in patients with NSCLC …": It does not sound right. If they are preclinical studies, they cannot show activity in patients ...The activity was shown in vitro in patient-derived cell lines and xenografts that carried *EGFR* ex20ins or *HER2* ex20ins or *HER2* amplification or *NRG1* fusions (ref. 96).

Reply 13: Thanks for the suggestion; we edited the sentence as suggested. See line 397-398

Line 430, "Pemigatinib is currently testing in various malignancies": it should be "Pemigatinib is currently being tested in various malignancies".

Reply 14: Thanks for the suggestion; we edited the sentence as suggested.

Line 435-437, “Preclinical studies showed no direct correlation between genetic alteration and sensitivity to the associated target drug and that not all cells respond equally to inhibitors”: Despite the authors claim in their rebuttal to the initial comments (reply 32), the sentence has not been changed and therefore remains unclear.

Reply 15: Thanks for the suggestion; we edited the sentence as suggested. See line 458-460

Table 5, abbreviation n.a: what does it mean? Is it the same as N/A in the other tables? If yes, the abbreviations should be unified.

Reply 16: Thanks for the comment. We uniformed the nomenclature as suggested

Line 455-456, “Unfortunately, only a tiny proportion of NSCLC have druggable mutations for which target treatments are approved”: It seems too reductive when one considers the approved inhibitors for the mutant forms of EGFR, KRAS, ALK, ROS1, BRAF, RET. Use a less reductive adjective such as “minor proportion” or similar.

Reply 17: Thanks for the suggestion; we edited the sentence as suggested.

References need thorough check and amendment: in reply 34 the authors state that they have corrected the references and that they are on line 480-820. However, they are on line 505-833 and still have several problems, including new ones:

- There are at least 22 incomplete references as they lack issue, page numbers and/or year of publication. Ref. 60 lacks the title
- Ref. 85: which journal or abstract book was it published in?
- Fonts and stile of references are not consistent.

Reply 18: Thanks for the suggestion; we edited references as suggested. See line 530-881