Peer Review File

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Reviewer A

This editorial piece gives a perspective on a research article published in J Clin Investigation by Malchers et al. The original research suggests that the specific configuration of FGFR1 amplifications present in a sub-set of squamous lung carcinomas is of importance in determining the sensitivity of these cancers to small molecule FGFR inhibitors. The research project stems from and tries to explain the observation that FGFR1 amplifications in general do not correlate well with the responsiveness to such inhibitors.
The editorial gives a brief overview of the genomic landscape of squamous lung carcinomas as derived by genomic studies and some key differences from the other main type of non-small cell cancers, adenocarcinomas. Then it provides some key results from the original research and briefly discusses the concept of bridge-fusion-breakage, which is believed to be at the root of the molecular events leading to 8p11-p12 amplifications. It concludes with some questions arising from the original research, the main concern being that the specific type of FGFR1 amplifications, that is tail-to-tail amplifications on or close to the FGFR1 locus, fail to predict inhibitor sensitivity in all cases. Authors suggest genomic heterogeneity of the configurations resulting from bridge-fusion-breakage events as a possible explanation and mention a possible role of other abnormalities in the 8p11 locus or elsewhere as possibly playing a role in the lack of correlation.

Comment 1: However, they do not mention the possibility that some or all of the amplified cancers are not dependent on deregulated FGFR1. In addition, due to the putative mechanism of the amplification, a more global disarray on the locus may deregulate the promoter or enhancer sequences of the gene by altering topological domains and alter transcription of the gene, which has been observed in genomic series.
Reply 1: We are grateful for the thorough review and insightful feedback on our draft. The patients with FGFR1-centered amplifications, but do not show the sensitivity to FGFR inhibitors, are mentioned in our manuscript (line 70 and 94, in revised manuscript) and also in other Editorial review by Mäkinen and Meyerson. We acknowledge that the potential mechanisms of FGFR1 amplification and molecular consequences, could be complex also involving epigenetic abnormality as suggested. We believe that this type of epigenetic abnormality is best presented with the example of enhancer-hijacking often seen in hematologic malignancies. We discuss this possibility in the revised manuscript.
Changes in the text: While Malcher et al. primarily examined the genetic structure of coding sequences related to FGFR1, there is a possibility that epigenetic abnormalities linked to chromosomal rearrangements affect the drug response. For instance, in cancer genomes, structural variations often result in enhancers being placed next to key driver genes (a process known as ‘enhancer hijacking’), which leads to transcriptional upregulation of cancer driver genes (16). One interesting phenomenone is that FGFR1 expression is more concordant with protein levels than FGFR1 copy numbers suggesting that roles of epigenetics needs to be further

investigated (17). (line 97-104, in revised manuscript).

Comment 2: It should be noted that the issue of J Clin Investigation that contains the original report includes also an editorial on it, which the authors of the current editorial fail to mention, despite that it contains unavoidably some common themes, given that both discuss the same results.

Reply 2: We recognize the issues raised. Despite the presence of some overlapping content, we believe that our manuscript uniquely covers topics such as advancements in sequencing technologies and pertinent perspectives. To ensure our readers are informed on these matters, we have incorporated the editorial commentary from Makinen and Meyerson (ref 21).

Comment 3: It would also be useful to discuss two recent articles (Voutsadakis IA. Life Sci. 2021;264:118729. doi: 10.1016/j.lfs.2020.118729. and Voutsadakis IA. J Clin Med. 2023;12(5):1711. doi: 10.3390/jcm12051711) on the 8p11 amplification which give a broader perspective on the amplicon and introduce the concept that amplifications of the locus occur in a significant minority of other cancers, and other genes may be drivers in these amplifications.

Reply 3: We appreciate the comments. The revised manuscript now includes a discussion of the separate comments, along with citations of the two suggested references (ref 18 and 19).

Changes in the text: Moreover, the amplification of 8p11.12, which involves *FGFR1* and additional genes such as *ZNF703, ERLIN2, PLPBP, ADGRA2, BRF2, RAB11FIP1, GOT1L1, ADRB3, EIF4EBP1, ASH2L, STAT, LSM1, BAG4, DDHD2, PLPP5, NSD3, LETM2*, and *TACC1*, is commonly observed in various tumor types, including breast, esophageal, and bladder cancers (18, 19). Although FGFR1 inhibitors have shown promising responses in some tumor types (20), further research is needed to understand the detailed genomic structure of 8q11.12 and the potential influence of other neighboring genes in different types of tumors. (line 104-110, in revised manuscript)

Some editing is needed in the manuscript. For example:

Line 26: Squamous cell lung… (Omit “The”).

Line 40-42: Meaning of the sentence somewhat unclear. Probably authors want to convey that anti-PD-1 therapies although targeting a specific inhibitor have no biomarkers for response in squamous Lung carcinomas. Phrase should be modified.

Line 54: Suggest: “A variable correlation between….”

Line 58: “contribute”.

Lines 111-113; Phrase unclear, please rewrite.

I suggest also that for medications that have now a clinical name, the name should be mentioned (e.g., BGJ398: Infigratinib, JNJ42756493: Erdafitinib) for clarity to clinicians.

Reply: We are grateful for the thorough review of our draft. The manuscript has been updated in response to your feedback, with the changes highlighted in yellow in the revised version.

Reviewer B

In the manuscript, the authors introduced the recent study by Malcher et al. (J Clin Invest 2023), which showed that the tail-to-tail rearrangements in or close to the FGFR1 gene lead to focal amplification of FGFR1 that may be a therapeutic target of FGFR inhibitors.
Overall, the manuscript is well-written, and I have a few comments that may help further improve the manuscript.

Comment 1. P2 lines 87-91 "Overall, tail-to-tail rearrangements in or close to FGFR1 were observed in 78% of the observed cases while only 25% of non-responsive cases showed the tail-to-tail rearrangements suggesting the tail-to-tail rearrangements as the distinguishing features of FGFR1 dependency." Were these "tumors (i.e., primary tumors from patients)" or "samples (including cell lines and PDX)"? If the latter was true, then "cases" may not be a suitable word.
Reply 1: We appreciate the comment. Those were aggregates of all the tumors/cell lines/PDX, and we revised the corresponding sentence as the following.
Changes in text: Overall, tail-to-tail rearrangements in or close to FGFR1 were observed in 78% of the observed responders (7 out of 9) while only 25% of non-responders (3 out of 12) showed the tail-to-tail rearrangements suggesting the tail-to-tail rearrangements as the distinguishing features of FGFR1 dependency. (line 68-71)

Comment 2. P3 lines 109- "Thus, the tail-to-tail rearrangement at or close to FGFR1 leading to the FGFR1-centered amplifications, indicate the FGFR1 dependency with the sensitivity to FGFR inhibitors." I'm afraid I don't think the previous paragraph and this sentence are linked together, and therefore, this sentence may not make sense.
Reply 2: We agree with the reviewer. We replaced "Thus," into "In summary,"

COMMENT 3. P3 line 125 "artificial intelligence-drive data" I assume this should be "artificial intelligence-driven data"
Reply 3: Corrected

4. P3 lines 128-129 "The reduced sequencing cost will bolster efforts for precision medicine, potentially leveraging insights whole-genome sequencing, …"
I find this sentence difficult to understand. Please check and consider rewriting.
Reply 4: We have revised the sentence accordingly.
Changes in text: The reduction in sequencing costs may benefit cancer genome sequencing by expanding eligibility for whole-genome sequencing, which provides detailed insights into characteristics associated with structural variations and chromosomal rearrangements. (line 116-119)