Author’s response to reviews

Title: Heterogeneous components of lung adenocarcinomas confer distinct EGFR mutation and PD-L1 expression

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Author’s response to reviews:

Response to Reviewer 1

I appreciate that Dr. Lucia Anna Muscarella provide important advice on our manuscript and I respond on behalf of our group to these comments by point-to-point.

Major comments:

Comments 1. Abstract. The last sentence was not really supported by the results of the manuscript. Response (see Result section, page 11, line 9-12): In the paragrpah of Result section“PD-L1 expression varied in LAC histological pattern and with tumor stage”. I add new information (seeing in the bracket) to account for the heterogeneous expression of PD-L1. Figure 1 is used to show the distinct expression in different histological components.

Comments 2. M&M section. Page 8 lines 17-23. The Authors described the ALK analysis, but no mention was made about this in the abstract and in the title of the paragraph. Please, clarify this point and add this informations. Response (see Abstract, last sentence in Results and M&M section, page 7, line 11): In a previous study (Clin Cancer Res. 2016 September 15; 22(18): 4585–4593), In an independent cohort of advanced, EGFR-mutant (N=68) and ALK positive (N=27) patients, PD-L1 expression was observed in...
24%/16%/11% and 63%/47%/26% of pre-tyrosine kinase inhibitor (TKI) biopsies using cutoffs of ≥1%, ≥5% and ≥50% tumor cell. In our study, 58.8%, 29.4% and 11.8% in ALK positive lung adenocarcinomas (N=17) were found PD-L1 expression tiered via cutoffs of &lt;1%, 1%-49% and &gt;=50% tumor cells (P &gt; 0.05). We didn’t discuss this result since that ALK positive lung adenocarcinomas were in a small sample and they had several histological types such as acinar, including cribriform and solid patterns. We couldn’t analyze these cases and discuss in more detail thereby, in addition to the restriction of words in abstract. We have added informations in corresponding paragraph (Results section, page 12, line 12-15).

Comments 3. Results. Through the manuscript (i.e. Title, Abstract and Results) the Authors generally reported an "EGFR mutation screening". However in the M&amp;M ("Digital PCR detection of EGFR mutations on LMD tissues" paragraph) they declared that only Ex19del, L858R and T790M mutations were investigated by ddPCR. Moreover, they not indicated the cut-off for positivity used to discriminate between positive and negative samples. Since we are talking about the tumor heterogeneity, please, reconsider any sections in light of these restricted molecular analysis and report positivity cut-off especially for naive T790M.

Response (see Methods section, from page 8, line 13 to page 9, line 3): In this study, We used a method that were reported in this article (R. Zhou et al. / Frontiers in Laboratory Medicine 2 (2018) 89–96) and referred to the methods by K Suzawa1 on ONCOLOGY REPORTS 37: 3100-3106, 2017. Here we added more detail on this method as follows:

A series of EGFR T790M mutation reference standards to set up cutoffs with the following mutation allele proportion of 0%, 0.1%, 1%, 10% and 50% were prepared using Human Genomic DNA, Female (Promega, US) and NCIH1975 Cell Line genomic DNA (Research DX, US). AS NCIH1975 cell line genomic DNA is heterozygous for EGFR T790M mutation, it is used as 50% EGFR T790M mutation reference standard without further processing. 0% EGFR T790M mutation reference standard is Human Genomic DNA, Female (Promega, US). 0.1%, 1% and 10% EGFR T790M mutation reference standard contains 0.2%, 2% and 20% NCIH1975 Cell Line DNA, respectively. 0% EGFR T790M mutation reference standard contains 20% NCIH1975 Cell Line DNA. The final concentration of the above reference is 20 ng/IL.

Comments 4. Results. "Patient Characteristics and Histopathological feature" section. Results about possible correlation of PD-L1 expression with ALK fusion were missed. Please, provide evidences about any results obtained about this.

Response (see Results section, page 12, line 12-15): In our study, 58.8%, 29.4% and 11.8% in ALK positive lung adenocarcinomas (N=17) tiered via cutoffs of &lt;1%, 1%-49% and &gt;=50% tumor cells (P &gt; 0.05). We didn’t discuss this result since that ALK positive lung adenocarcinomas were in a small sample and they had several histological types such as acinar, including cribriform and solid patterns. We couldn’t analyze these cases and discuss in more detail thereby, in addition to the restriction of words in abstract. We have added information in corresponding paragraph.

Minor Comments:

Comment 1. Genes name must be written in Italic.
Response: Genes name has been rectified as requested.

Comments 2. Some grammatical errors should be corrected
Response: Language of this manuscript has been polished.
Response to Reviewer 2

I appreciate that Dr. Giulio Rossi provide important advice on our manuscript and I respond on behalf of our group to these comments by point-to-point.

Comments: The only point of discussion relies on the references that should be mentioned in the paper. There are important works published in literature that should be cited among the references, as Cai W et al JCO 2015, Zito-Marino F, et al Am J Surg Pathol 2019 among others.
Response (see references 21 and 26 added in Discussion section):

Yes. There are some important studies have been done in this respect. Cai W et al. have reported intratumoral heterogeneity in ALK/EGFR coalterd lung adenocarcinomas. The fact that lung adenocarcinomas harbor both ALK fusion and EGFR mutation suggests that subpopulation of cancer cells have really heterogeneously biological features. Zito-Marino F, et al observed that PD-L1 expression was more frequently in ADC with solid pattern as reported in this study. In addition, they found all PD-L1-positive cases were epidermal growth factor receptor wild-type as we noticed in components of lung adenocarcinomas. These reference have been added in our manuscript.

Best wishes

Yi-Ran Cai