**Peer Review File**

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**Reviewer A**

*Lung cancer is a leading cause of death worldwide, thus establishing and finding new markers, especially non-invasive markers, which can be obtained from a primary tumor diagnosis is crucial and might help in appropriate management of NSCLC patients. However, authors are required to improve their manuscript and provide some substantial changes.*

*Comment 1: Abstract section: please explain all abbreviations used in this section, for example (but not limited to) ICI, FDG PET/CT or SUVmax and SUVmean are not explained.*

**Reply 1: We apologize for overlooking the explanation of all abbreviations. In the revised manuscript, we have addressed these details. We appreciate the Reviewer for their helpful suggestions. (Pages 2-3)**
**Changes in the text: lines 24 " immune checkpoint inhibitor (ICI)", lines 26-28"of 18F-fluorodeoxyglucose (FDG) uptake on positron emission tomography/computed tomography (PET/CT) and clinical features in non-small cell lung cancer (NSCLC) ", lines 42-43"Two clinical features and two PET parameters were identified through lasso regression and logistic regression analysis including pathology type, cancer antigen 125 (CA125) level, maximum standardized uptake value (SUVmax), and metabolic tumor volume (MTV)."**

*Comment 2: Abstract, methods section: please add information about which parameters were assessed in manuscript.*

**Reply 2: Thanks to the reviewer for the suggestion. We have modified our text as advised (Pages 2, lines 34)**
**Changes in the text: add "Twelve clinical features and five PET parameters were assessed."**

*Comment 3: Keywords: please explain all used abbreviations in this section.*

**Reply 3: Thanks for the reviewer's comments. We have modified our text as**

**advised (Page 3, lines 31-32)**
**Changes in the text: "18F-fluorodeoxyglucose uptake on positron emission tomography/computed tomography($^{18}$F-FDG PET/CT), maximum standardized uptake value (SUVmax),"**

*Comment 4. Methods, Image analysis section: while using an open-source software like LIFEx, as specified in the license, when using LIFEx to produce results included in a paper, poster or presentation, please cite: "C Nioche, F Orlhac, S Boughdad, S Reuzé, J Goya-Outi, C Robert, C Pellot-Barakat, M Soussan, F Frouin, and I Buvat. LIFEx: a freeware for radiomic feature calculation in multimodality imaging to accelerate advances in the characterization of tumor heterogeneity. Cancer Research 2018; 78(16):4786-4789".*

**Reply 4: We apologize for the neglected reference, and we have added citations to this reference in the revised manuscript. (Page 7, line 101, Page 16, lines 312-315)**
**Changes in the text: Page 7, line 117"[14]". Page 16, line 312-315"[14]C Nioche, F Orlhac, S Boughdad, S Reuzé, J Goya-Outi, C Robert, C Pellot-Barakat, M Soussan, F Frouin, and I Buvat. LIFEx: a freeware for radiomic feature calculation in multimodality imaging to accelerate advances in the characterization of tumor heterogeneity. Cancer Research 2018; 78(16):4786-4789".**

*Comment 5: Methods, Image analysis section: based on which features authors selected which tumor from multiple tumor patients was used in the analysis? It was a tumor with the biggest SUV? Biggest diameter? It should be clearly stated in this section.*

**Reply 5: Thanks for the reviewer's question. Actually, we have explained this issue in the original text. See page 7 lines 108-109"For patients with multiple lesions, MTV and TLG included only the single primary lesion used for TMB detection, excluding other primary and metastatic lesions." In other words, for patients with multiple lesions, we analyzed the lesions that were used for TMB detection. Since our study is retrospective, we first identified which lesion was used for TMB detection, and then analyzed the PET metabolic parameters of that specific lesion. This approach ensures that the correlation between TMB and SUV corresponds to the same lesion, thereby enhancing the credibility of our conclusions. Perhaps the original text was not expressed clearly. We have modified our text as advised. (see Page 7, line 118-120)**
**Changes in the text: add "For patients with multiple lesions, only the single primary lesion used for TMB detection was included in the image analysis,**

**excluding other primary and metastatic lesions."**

*Comment 6: Results, Predictive model and evaluation of model section: the ROC and AUC abbreviations are already explained in the material and methods section.*

**Reply 6: Thanks for the reviewer's comments. We have modified our text as advised. (see Page 10, line 181)**
**Changes in the text: "The predictive performance of the model was assessed using ROC curves. The AUC for the model was 0.822 (95% CI, 0.751–0.894) in the training set and also 0.822 (95% CI, 0.731–0.912) in the validation set (Fig. 5). "**

*Comment 7 : Results section: Does authors performed an analysis based on histopathology and compared TMB between SCC and ADC? Additionally, comparison of TMB and all FDG PET parameters should be performed within SCC and ADC group, which will increase the value of the manuscript.*

**Reply 7: Thanks for the reviewer's comments. Thank you for your valuable suggestions. Indeed, comparing FDG PET parameters and TMB within each pathological group (SCC and ADC) can increase the value of the manuscript. However, the primary focus of this article is to establish a TMB prediction model. Based on our variable selection results, both PET parameters and pathological types are predictive factors for TMB levels. Therefore, it is not necessary to analyze the correlation between PET parameters and TMB levels within each pathological group at this time.**

**We believe that including these additional analyses might divert the focus from the main objective of our study and complicate the interpretation of the model. To maintain the clarity and focus of our research, we have decided to reserve the analysis of the correlation between PET parameters and TMB levels within each pathological group for future studies.**

**In future research, we plan to specifically analyze the correlation between PET parameters and TMB levels within different pathological groups, such as SCC and ADC. This approach will involve conducting in-depth analyses within each pathological group to understand the unique relationships between PET parameters and TMB levels. By adopting this strategy, we aim to systematically and thoroughly investigate these correlations, thereby enhancing the overall value and impact of our research.**

**Changes in the text: none.**

*Comment 8: Discussion section: ICIs are already, earlier explained in the Introduction section, thus there is no need to explain it once again.*

**Reply 8: Thanks for the reviewer's comments. We have modified our text as advised (see Page 11, line 193)**

**Changes in the text: Page 12, line 227-228：“Consequently, identifying effective predictive biomarkers for ICIs is critical to improving prognosis in NSCLC patients ”**

*Comment 9: Discussion section -> this whole section should be changed and rewritten. Even if there is a small number of publications similar to the one which authors performed, they should compare their results with others (even if the group of patients is smaller compared to their study).*

**Reply 9: Thanks for the reviewer's comments. Indeed, this is an oversight on our part. To our knowledge, this is the first study to construct a TMB level prediction model for NSCLC patients using PET parameters and clinical features. The foundation of this study is the correlation between PET parameters and TMB levels. Previous studies on the correlation between PET parameters and TMB levels are limited. Moon et al. investigated the correlation between PET parameters and TMB levels in lung cancer patients and found no significant relationship between SUVmax and TMB. However, their study overlooked a crucial issue: they did not account for the time interval between the [18F]FDG PET-CT and biopsy. A longer interval could lead to a poor correlation between SUVmax and TMB. Amin Haghighat Jahromi et al. later addressed this issue and demonstrated a significant positive correlation between SUVmax and TMB in multiple advanced cancers, limiting the interval between the [18F]FDG PET-CT and biopsy to within six months. Qian Zhang et al. conducted a more in-depth study on the correlation between PET parameters and TMB levels in NSCLC patients and confirmed that the SUVmax values were higher in the high TMB group compared to the low TMB group.**

**Compared to previous studies, our study has the following improvements: a. We have more strictly limited the interval between [18F]FDG PET-CT and biopsy to within three months, ensuring temporal consistency between PET parameters and TMB levels. b. We have expanded the sample size. c. For the first time, we have**

**used PET parameters and clinical features to establish a prediction model for TMB levels. d. We explored different cutoff values for TMB levels, as there is no uniform cutoff value for high and low TMB groups. Qian Zhang et al. used TMB > 10 for grouping in their study, but this is not suitable for constructing a prediction model because the majority of patients have TMB levels less than 10. Based on previous research, we used 4 as the cutoff value between high and low TMB groups, making the high TMB group and low TMB group more balanced and enhancing the credibility of the prediction model. We have modified our text as advised**
**Changes in the text: (see Page 14, lines 266-277)**

*Comment10. Figures: please explain all used abbreviations in the figures. A lot of are not explained, especially in figure 2 and 3.*

**Reply 10: Thank you for your valuable comments on our manuscript. Regarding the abbreviations used in the figures, we have added explanations for all abbreviations in the legends, especially for Figures 2 and 3.**
**Changes in the text: Page 30, lines 506-516: LM, lymphatic metastasis; PT, pathology type; FMTH, family malignant tumor history; MPLC, multiple primary lung cancer. LM, lymphatic metastasis; PT, pathology type; FMTH, family malignant tumor history; MPLC, multiple primary lung cancer. ROC, receiver operator characteristics; AUC, area under the curve.( Abbreviations that have already been explained in the previous text or table will not be repeated)**

**Reviewer B**

*The authors report on the development of a predictive model for TMB status that employs a selected number of features derived from 18F-FDG PET, tumor marker and histological subtype. The study is well designed, the methodology is sound and the number of patients used for training and validation is quite large. The manuscript closes with a nomogram which would potentially allow to use these findings in a clinical routine setting.*

*Comment 1: The characteristics of TMB high and low groups are described, please also describe these characteristics for training and validation cohort (in supplementary tables) to demonstrate random selection*

**Reply 1: Thanks for the reviewer's comments. Based on your suggestion, we have added a new supplementary Table 1 in the supplementary materials to detail the**

**balance between the training and validation cohorts. In this table, we describe the main characteristics of the training and validation set to demonstrate the randomness and balance of our sample selection. Supplementary Table 1 includes key clinical parameters for each group to ensure there are no systematic biases between the different groups.**

**Changes in the text: Page 10, line 174-178, add: In Supplementary Table 1, we provide a detailed description of the distribution of various metrics in the training and validation set. The results indicate that there are no statistically significant differences between the two groups for these metrics. Therefore, the data from both cohorts are free from systematic bias and are suitable for model development and validation.**

*Comment 2: what is the underlying hypothesis for 18F-FDG to relate to TMB? among the mutations that are observed in TMB-high, many will not have a direct link to tumor metabolism/glycolytic pathways. Please postulate in the Discussion how 18F-FDG uptake is linked to TMB.*

**Reply 2: Thanks for the reviewer's comments. Based on the previous study, we propose that an elevated mutation burden, indicated by TMB, could be linked to metabolic remodeling and immune inflammatory response. These characteristics, in turn, may correlate with increased SUVmax. Of course, we are only briefly stating this hypothesis in the article, as our research focuses on the construction of predictive models, rather than delving into the mechanism of the correlation between TMB and PET parameters. We have modified our text as advised (see Page 12, line 215-217)**

**Changes in the text: see Page 12, line 215-217, add. "Based on the previous study[10], we propose that an elevated mutation burden, indicated by TMB, could be linked to metabolic remodeling and immune inflammatory response. These characteristics, in turn, may correlate with increased SUVmax."**

*Comment 3：If available, please consider to validate the nomogram on an independent dataset of n=100-200 patients (from another hospital? preferably with another PET scanner) and demonstrate its accuracy for predicting TMB status. This would greatly enhance the impact of this work.*

**Reply 3: Thank you for your valuable comments. We understand the importance of validating the nomogram on an independent dataset, which would indeed enhance the impact of our research. However, due to various reasons, we are**

**currently unable to obtain a new dataset for independent validation. Specifically, we faced limitations in accessing additional data due to time constraints and the unavailability of suitable external collaborations at this moment.**

**Despite these limitations, we have ensured that our current dataset underwent thorough and rigorous validation to demonstrate the reliability and accuracy of our results. We performed extensive internal validation using cross-validation techniques to confirm the robustness of our model.**

**Moreover, we are actively seeking opportunities to collaborate with other institutions to obtain new datasets for future studies. We plan to validate our nomogram on an independent cohort as soon as it becomes feasible. This will be a critical part of our ongoing research efforts to further substantiate our findings.**

**Changes in the text: None.**