Peer Review File

Article information: http://dx.doi.org/10.21037/tcr-20-480

Reviewer A's comments:

1. *The frequency of BRAF mutations is low 1,8%. BRAF V600E it is 3% and non-V600 BRAF mutations are around 8%. In total BRAF mutations in NSCLC is around 10-11%, as described in reference 11.*

Response: Thank you for your comments. BRAF mutations occur in 2-4% non-small cell lung cancer (NSCLC) patients that have been reported in several studies (please see below). The BRAF mutation screening in our study is taken from a large cohort(N=3669), and the frequency of BRAF mutations 1.8% which is very close to 2-4% reported in previous studies.

[1]. Lin Q, Zhang H, Ding H, Qian J, Lizaso A, Lin J, Han-Zhang H, Xiang J, Li Y, Zhu H. The association between BRAF mutation class and clinical features in BRAF-mutant Chinese non-small cell lung cancer patients. J Transl Med. 2019 Aug 30;17(1):298.

[2]. Baik CS, Myall NJ, Wakelee HA. Targeting BRAF-Mutant Non-Small Cell Lung Cancer: From Molecular Profiling to Rationally Designed Therapy. The oncologist 2017;22: 786-96.

[3]. Luk PP, Yu B, Ng CC, Mercorella B, Selinger C, Lum T, Kao S, O'Toole SA, Cooper WA. BRAF mutations in non-small cell lung cancer. Translational lung cancer research 2015;4: 142-8.

2. *Some key references are not mentioned as Planchard et al, Lancet Oncol 2017 with targeted therapy for BRAF V600E with dafrafenib plus trametinib. In the manuscript, there is no reference to BRAF and MEK inhibitors.*

Response: Thank you for your valuable comments. We have cited "*Planchard et al, Lancet Oncol 2017 with targeted therapy for BRAF V600E with dafrafenib plus trametinib*" as a reference.

3. *Non-V600 BRAF mutations are sensitive to the combination of BRAF or MEK inhibitors plus SHP2 inhibitors. See reference 11 (Bracht et al. Cancers 2019).*

Response: Yes, we agree with your opinion. However, the sensitivity of targeted therapy in non-V600 BRAF and V600 BRAF is very different. Based on the mechanism of activation, kinase activity, and sensitivity to inhibitors, a functional mutation classification system for BRAF mutations has been recently introduced. According to functional class, RAS-

independent kinase-activating V600 monomers are categorized as class 1; RAS-independent kinase-activating dimers that are resistant to vemurafenib are categorized as class 2; RAS-dependent kinase-inactivating heterodimers are categorized as class 3. Class 1 BRAF gene change-V600 mutant kinase activating monomer BRAFV600E/D/K/M/R mutation which have the highest kinase activity, and they act against BRAF monomer inhibitors (verofenib, dabrafenib, and konafinib) or MEK inhibitors (cobinitinib, bimetinib, and trametinib) or the combination of those two is sensitive. Class 2 BRAF gene change-kinase-activating dimer BRAF P367L/S, G464E/V, G469A/V/R, L485W, N486_A489delinsK, N486_P490del, E586K, L597Q/R/S/V, T599T/S, T599I/K, K601E/N/T, and K601_S602delinsNT mutations, BRAF kinase domain replication, and BRAF fusion. Class 2 BRAF mutations are sensitive to BRAF dimer inhibitors (Lifirafenib, LY3009120, and LXH254) or MEK inhibitors or a combination of both. Class 3 BRAF gene change-kinase inactive heterodimer BRAF D287H, V459L, G466A/E/V, S467L, G469E, N581I/S/T, D594A/G/H/N, F595L, G596D/R mutation. This type of BRAF mutation is not a driver gene, BRAF inhibitor ± MEK inhibitor or BRAF dimer inhibitor ± MEK inhibitor or MEK inhibitor is ineffective.

4. *Chemotherapy has limited effect including pemetrexed combination. See again Bracht et al. Cancers 2019, where also patients were receiving chemotherapy and no differences were noted for those treated with pemetrexed.*

Response: Yes, we agree with you that chemotherapy had shown limit efficacy in Bracht et al.'s study. However, in their study, patients with BRAF mutation and received chemotherapy is less than 10 patients. The sample size and data are too limited to conclude the author written in the discussion session. The efficacy of chemotherapy is still controversial in NSCLC patients with BRAF mutations. More studies are still needed to address this question.

5. *The discussion is too long.*

Response: Thank you for your suggestion. We have our discussion shortened in the revised manuscript.

Reviewer B's comments:

This is a tremendous effort to have looked at so many patients for BRAF mutations and to have collected data on this large group. There are many issues with this manuscript though.

1. *The abstract is incredibly long and essentially covers the entire paper. It must be shortened to be meaningful to the reader.*

Response: Thank you for this comment. We completely agree with this point and have shortened to be meaningful as possible as we could.

2. *In the introduction, this is not necessarily true: Advanced lung cancer has always been the leading cause of cancer death in all populations worldwide.*

Response: Thank you for this comment. In response, we have rewritten this sentence "Lung cancer has always been the leading cause of cancer death in all populations from worldwide" which is consistent with the reference.

3. *The introduction could be more linear and should have a better discussion of targeted therapy and at least a mention of immunotherapy. Immunotherapy needs to be included in the discussion as well.*

Response: Thank you very much for your comments. The main purpose of this study is to observe the association between the functional classification of BRAF mutations and the survival benefit of pemetrexed-based chemotherapy. In that case, we assume that the discussion about targeted therapy and immunotherapy would not be necessary. However, if the editors also suggest us to add relative information in the discussion section, we would like to consider this again.

4. *This is retrospective what is the frequency of the scans? How was RECIST done if scan frequency varied? Did the authors go back and do formal RECIST measurements on all the scans for the 41 patients in this analysis?*

Response: We agree with your suggestion that we have rewritten as follows: "Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST v1.1) [22]. The treatment response was evaluated by CT scans at the baseline of initial therapy and every 6 weeks thereafter." In lines 112-114.

5. *The authors state that written informed consent was obtained from each patient to use the clinical data for research before the medical intervention started (though they were*

getting chemotherapy and this was not randomized and the BRAF testing results would not likely have returned before the patients started chemotherapy?). I want to clarify that every patient was consented to start back in 2014 before they received any chemotherapy. If that is true why did the authors not do a randomized study? For a retrospective study, it is rare that patients are consented before start of any therapy. Please clarify in the methods section. "This study was approved by XXXXXX (author identities are concealed for peer review) Ethics Committee and written informed consent was obtained from each patient to use the clinical data for research before the medical intervention started."

Response: Thank you for your comments. We have clarified the statement "This study was approved by the Chinese Academy of Sciences University Cancer Hospital (Zhejiang Cancer Hospital) Ethics Committee and written informed consent was obtained from each patient to use the clinical data for research before the medical intervention started." In the methods section. This is a retrospective study and we have followed the regular procedure for every clinical research by abstaining patients' written informed consent before research.

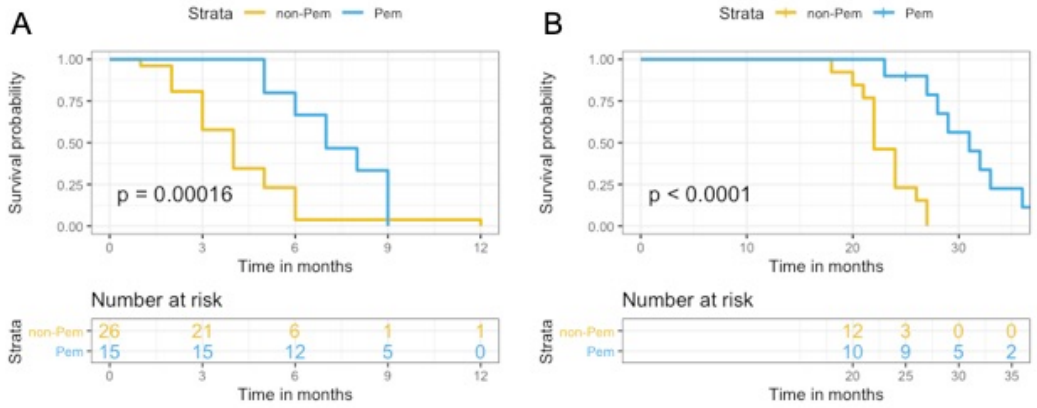
6. *37 never smokers are very high, especially with only 5 female patients.*

Response: We agree with this observation. Indeed, according to literature, most NSCLC patients with a BRAF mutation were men and current or former smokers. However, this is a real-world study and we have already screened those patients from a large cohort population. It is not uncommon to have male patients with no smoking history in the clinic. As we discussed in our manuscript, "up to now, no convincible clinical feature could help identify patients harboring BRAF mutations [24]". Therefore, a larger sample size could be helpful to address this discrepancy in the future.

7. *The analysis is very confusing as in some places only the first-line regimens are compared and in others, pemetrexed at any time point is discussed. It seems that only 3 patients have pemetrexed beyond the first line so I would suggest the authors remove the analysis of pemetrexed at any point and only look at first-line therapy for the comparisons. Otherwise, it is very confusing as it is not okay to mix 1st/2nd line regimens when calculating ORR and DCR. These should be reported separately with the fist line combination regimens versus 2nd line single-drug regimens. This requires re-doing the calculations that are in figure 3A and in the text on lines 137-140 or explaining this more fully as it is very confusing what are the first line comparisons and what is looking at all lines.*

Response: Thank you for this valuable suggestion. We would like to follow your suggestion and corrected the calculating ORR and DCR only in first-line therapy. In the raw data, two patients accepted both 1st/2nd line regimens containing pemetrexed for an unknown reason, that is why the number of patients looks like a little bit confusing. To be

clear, we focus on evaluating the efficacy of pemetrexed based regimen only in 1st line since there were few patients in the 2nd line. We have redrawn Figure 3 accordingly.



8. *It seems that no patients had any BRAF targeted therapy which is confusing to me as this was done from 2014-2019 and there is the availability of BRAF agents. If any patients did get them this needs to be discussed.*

Response: Thank you for this valuable suggestion. According to the record, all patients with BRAF mutations have not received any BRAF targeted therapy during our study. Although BRAF agents have been approved several years ago in China, they are very expensive and have not been covered by medical insurance. Most of the patients could not endure the high expense of those targeted drugs. That is the main motivation that encourages us to find evidence of better and acceptable treatment for those patients.

9. *The OS data is okay to present regardless of when the pemetrexed was given, however, was histology and other factors include in the multivariate analysis? The multivariate analysis as presented is very confusing.*

Response: Thank you for your comments. We have multivariate analysis included histology and other factors as you mentioned above as well as the pemetrexed treatment factor. Considering the potential confusing presentation, we also adjusted Table 4 accordingly.

Table 4 Univariate and multivariate analysis of 41 NSCLC patients with *BRAF* mutation.

variable	category	PFS analysis						OS analysis					
		Univariate			Multivariate			Univariate			Multivariate		
		HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value	HR	95% CI	P value
Age, years	≤60/>60	1.35	0.59-3.06	0.476	-	-	-	1.08	0.47-2.47	0.86	-	-	-
Sex	Male/female	1.54	0.54-4.41	0.422	-	-	-	0.77	0.3-2	0.589	-	-	-
Smoker	Yes/no	0.83	0.3-2.35	0.73	-	-	-	0.72	0.25-2.03	0.533	-	-	-
Stage	IIIB/IV	0.73	0.33-1.59	0.424	-	-	-	0.67	0.3-1.47	0.312	-	-	-
Histology	Adenocarcinoma/Non-adenocarcinoma	0.42	0.2-0.88	0.022	-	-	-	0.5	0.24-1.04	0.064	-	-	-
First-line chemotherapy	Pemetrexed-based/Non-pemetrexed	0.31	0.15-0.61	**<0.001**	0.16	0.05-0.47	**<0.001**	0.28	0.13-0.61	**0.001**	0.29	0.1-0.82	**0.02**
BRAF mutant Group	Class 1/Class 2/Class 3/Non-class 1-3	0.96	0.75-1.23	0.735	-	-	-	1.53	1.2-1.95	**<0.001**	2.15	1.47-3.17	**<0.001**

10. *Grammatical corrections are needed throughout. For instance, "About 2-4% of patients with BRAF mutation have been detected in advanced NSCLC patients from western countries [8, 9]." This would be better written as "BRAF mutations have been detected in approximately 2-4% of advanced NSCLC patients from western countries." Also in the following sentence, the word "harvested" is not correct and you could just say "had". "Patients with class 1 BRAF mutation also harvested longer OS than others after chemotherapy." There are many other examples.*

Response: Thank you for this valuable suggestion. We have tried our best to correct the writings as your suggestion.
1) "BRAF mutations have been detected in approximately 2-4% of advanced NSCLC patients from western countries."
2)" Patients with class 1 BRAF mutation also had longer OS than others after chemotherapy."
And we also did our best to correct the rest of our manuscript.

11. *Line 180 is incorrect.: "Platinum-based chemotherapies were not effective in metastatic NSCLC patients with BRAF V600E [24]." Perhaps in that one paper, but many other studies contradict that statement and even here the PFS for first-line chemotherapy was not very different than many trials.*

Response: Yes, we agree with your comment. There is no convincible conclusion of which kind of regimen is better in metastatic NSCLC patients with BRAF V600E. We have deleted this sentence already.

12. *Did the 5 driver mutation patients skew the results? Which group were they in? This is somewhat addressed in the tables.*

Response: Thank you for this valuable suggestion. Mutations of Oncogenes are usually mutually exclusive[1], eg. EGFR, KRAS and BRAF[2]. In our study, only 5 driver mutations have been found and their distribution in groups were as follows: EGFR (n = 3, 2 in class 1 and 1 in non-class1,2,3), KRAS (n = 1) in class 3, EML-ALK (n = 1) in others (non-class 1,2,3). We also redo the analysis after removing those patients and the conclusion is the same. Therefore,
The inclusion of those patients has no significant impact on the results.

[1] Cisowski J, Bergo MO: What makes oncogenes mutually exclusive? Small GTPases 2017, 8(3):187-192.
[2] Paik PK, Arcila ME, Fara M, Sima CS, Miller VA, Kris MG, Ladanyi M, Riely GJ: Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. Journal of clinical oncology 2011, 29(15):2046.

13. *There is an error in this reference with a lot of symbols: Line 252: 15. Cardarella S, Ogino A, Nishino M, Butaney M, Shen J, Lydon C, Yeap BY, Sholl LM, Johnson BE, J.nne PA. Clinical, Pathologic, and Biologic Features Associated with BRAF Mutations in Non–Small Cell Lung Cancer. Clinical Cancer Research 2013;19: 4532.*

Response: Thank you for this comment. We have corrected this reference" Cardarella S, Ogino A, Nishino M, Butaney M, Shen J, Lydon C, Yeap BY, Sholl LM, Johnson BE, Jänne PA. Clinical, pathologic, and biologic features associated with BRAF mutations in non-small cell lung cancer. Clinical Cancer Research 2013;19: 4532."

14. *Figure 3 C is very unusual. I cannot believe that no patients died until 20 months. Can the authors confirm this? Figure 3 needs to have the N at each time point for the number at risk.*

Response: Thank you for the comment. Yes, we have double-checked our data and made corrections accordingly. However, for Figure 3C, this is the originally collected data and the reason why no patients died until 20 months is unknown. Perhaps those patients with class 1 BRAF mutation carriers may have a better outcome than we could expect. Larger sample collection and longer follow-up could be an important way to find the answer. The N at each time point for the number at risk has also been added in the revised Figure 3.

15. *Per table 3 there are differences in the groups. It may not have reached statistical significance, but this study needs multivariate analysis, particularly in regards to the gender balance, histology, and most important stage. Class 1 is 70% adenocarcinoma, but the others vary. Also, the staging is very different. 83% of the class I are stage IIIB, but 40% or zero of the other groups are stage IIIB. I cannot believe it is not statistically different that 82.6% of class I are stage IIIB and 100% of other is stage IV.*

Response: Thank you for your comments. Yes, we agree with you that the potential imbalance in baseline among groups. However, we have confirmed our results by our clinical statistician. To limit the conclusion that could be influenced by those baseline factors, we have already performed the multivariate analysis and reconfirmed the results in the revised Table 4.

16. *In the first-line chemotherapy comparison, there are only 15 patients who had pemetrexed and 10 of them were class 1 (67%) but 13/26 (50%) of the non-pemetrexed therapy was given in the class I group. So there is an imbalance with more of the class I (who we know do better overall from this and other analyses) in the pemetrexed first-line group and fewer in the non-pemetrexed first-line group.*

Response: Thank you for this valuable suggestion. As we mentioned above, this is a retrospective study that the imbalance of the distribution of patients into different groups could not be avoided. However, we still could get useful information by showing the trend of the conclusion. Enlarge the sample size in the future could be the way to better balance those baseline characteristics.

17. *Per table 3: 15 patients had pemetrexed based chemotherapy first line and 26 had non-pemetrexed based chemotherapy first-line. In second-line therapy, only 3 patients had pemetrexed and 38 had non-pemetrexed therapy. But in the text under systemic therapy (starting line 130) it states that ALL patients received first line-chemotherapy regimen with 18 getting pemetrexed/platinum and 7 getting pemetrexed monotherapy (which would be 25 patients) and only 16 got non-pemetrexed based chemotherapy. The authors state that the ORR was 36% (9/25) and the ORR was 25% (4/16 for the other group). The authors must explain why table 3 and the text do not match.*

Response: Thank you very much for your comments. We should avoid this error before we present our paper. Thanks to your observation, we have immediately corrected those data in the revised manuscript accordingly.
"All patients received a first-line chemotherapy regimen, including pemetrexed/platinum (n=13), pemetrexed monotherapy (n=2), paclitaxel/platinum (n=3) and gemcitabine/platinum (n=19), others(n=4)."
"The ORR and DCR of pemetrexed-based chemotherapy were 33.3% (5/15) and 53.3% (8/15), respectively. The ORR and DCR of the other chemotherapy regimens were 26.9% (7/26) and 42.3% (11/26), respectively. The median PFS (mPFS) for the 15 patients who received pemetrexed-based chemotherapy was 7.5 months, while the mPFS of the 26 patients who received other chemotherapy regimens was 4 months ($P < 0.0001$, HR=0.3(95%CI, 0.16-0.58); Figure 3A)."

18. *Stage IIIB in the 7th edition staging this would include N3 or T4 but would NOT include malignant effusions. Standard therapy for stage IIIB includes radiation therapy for the vast majority of patients and not chemotherapy alone. In this study, 23 patients (more than half) are stage IIIB with a significantly improved prognosis versus stage IV and 19/23 (83%) are in the class I group. The authors need to do a better job of proving that this imbalance is not the cause of some of the discrepancies.*

Response: Thank you for this valuable suggestion and we agree with your professional comments. The standard therapy for stage IIIB is concurrent chemotherapy and radiotherapy and followed by immunotherapy in selected patients. In China, it is estimated that only 20-30% of patients in stage IIIB could tolerate and complete the concurrent chemotherapy and radiotherapy. Maybe due to the poor health condition and short of financial support. Therefore, most of the patients in stage IIIB have to receive similar therapy like stage IV in reality until now. Based on the above situation, we convinced that the imbalance of the stage would not cause obvious discrepancies.

19. *This is an interesting and novel analysis, but there are many major concerns that I have outlined in the comments to the authors. In particular, a statistician needs to look at the multivariate analysis as I have concerns about the methods and results, but I am not a statistician myself. If the authors can address the comments the paper may be publishable.*

Response: Thank you for this valuable suggestion. We have tried our best to work out all your professional questions. Thank you again for your thoughtful review of this manuscript, as well as your continued consideration of this manuscript for publication