Peer Review File

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Reviewer A

Thank you for providing your work about exploring the efficacy of immunotherapy in NSCLC patients with BRAF mutations. These case series can be interesting for readers and contribute to provide the real-world status of treating such patients.

Abstract

Line 14: According to NCCI v. 5.2024 it may also be used as a first line nscl.pdf (nccn.org)

Reply: Text updated according to suggestion

Changes in the text: Line 24

Introduction

Line 35: Please explain “impaired development”? Development to which aim?

Reply: Impaired kinase development is explained in the text line 42. Class III mutations occurs in other codons than 600 an results in impaired kinase development in contrast with class I and II mutations that causes kinase activation.

Changes in the text: no changes

Line 51: Please rephrase to "smokers”

Reply: Updated.

Changes in the text: Smokers, line 59

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Please improve analysis of the cases and describe all cases more consistently including other features (performance status, co-morbidities, smoking status) and all molecular co-alterations beyond BRAF-mutations (if any?) found by NGS, providing also the name of the panel/panels used, and PD-L1 status. If different panels were used, please underline that it may detect patients with different molecular configurations.

Reply: Smoking status is presented in table1. Line 342. Co-morbidities if known and significant are described in each case report section. Eg . Case 2 line 137-138, Case 4 lines 160-161. All found co-mutations are also presented in the description of case 1 and 6. Reagarding NGS panel it is described in detail in methods section line 96-97. Method of PD-L1 test was added.

Changes in the text: Added line 160, 174 , 198, ECOG added. Co- mutations were added to the table 1 . Method of PD-L1 test was added line 106

Line 130: Please add: "...and the TNM stage (please provide)"

Reply: Provided as suggested.

Changes in the text: Stage added line 142 and 151

Line 139: Why five cycles were given? Any data which supports more than 4 cycles?

Reply: Further explained in the text.

Changes in the text: Line 153 : 4 cycles of platinum dublet chemotherapy and one

cycle of pemetrexed maintenance monotheraphy.

Line 142: Please specify when the NGS was performed? Was it performed on diagnostic biopsy or rebiopsy?
Reply: NGS was performed from archwie tissue (biopsy) at the time of progression.
Changes in the text: Added information line 103

Line 143: Was it a single mutation or there also were co-alternations?
Reply: In each case co-alternation were described if found.
Changes in the text: Co- alternations added to summary table 1.

Line 149: please refer to question at line 139.
Reply: Explanation added in the text.
Changes in the text: Line 163 : 4 cycles of platinum dublet chemotherapy and one cycle of pemetrexed monothereaphy.

Line 171: "The resected tumor..." - please rephrase to "The resected brain tumor"
Reply: Modified as advised
Changes in the text: Line 182 brain added.

Discussion
Line 215: please develop abbreviation of "IL" as you with other abbreviations.
Reply: Changed as advised.
Changes in the text: Line 225 Interleukin- 1

Line 222: Please underline what are the consequences of this observation. Are there any similar data regarding other NSCLC biomarkers, making therefore BRAF more unique among other NSCLC biomarkers?
Reply: In the discussion there are several biomarkers mentioned eg. KRAS, MET and HER 2 with reference to IMMUNOTARGET.
Changes in the text: Added line 287

Line 227, 229-230: please adjust the font.
Reply: Changed as advised.
Changes in the text: Lines 230-236

Conclusions
Line 328-329: "The sequence of immunotherapy and targeted therapy in BRAF-mutated patients is justified" - do you mean the sequence of treatment with first line immunotherapy and second line BRAF/MEK-inhibitors? Treatments might be justified, but do we have enough data for sequence of treatments?
Reply: Rephrased as suggested.
Changes in the text: Added line 328.

Line 334-339: "Despite advancements in NSCLC treatment, the study indicates a need for continued research into more effective and tolerable treatment options, especially for patients with rare or complex genetic profiles. Future studies with larger cohorts are necessary to validate the role of immunotherapy as therapeutic

option for NSCLC patients harboring BRAF mutations "- please remove or rephrase the sentences as they do not contribute to answer your topic issue of exploring efficacy of immunotherapy.
Reply: Removed as suggested.
Changes in the text: Removed line 333-336

Additional comments:
1. In the discussion (lines 220-238) considering the use of immunotherapy after BRAF/MEK-inhibitors in NSCLC-patients with BRAF mutations, are there any issues regarding toxicity of such sequence?
Reply: Reference regarding toxicity added.
Changes in the text: Added line 244-248

2. Data from literature and your work point out that patients with BRAF non-V600E (ORR 34%) mutations respond slightly better to immunotherapy than patients with BRAF V600E mutation (ORR 20%) (Chen, J., Lu, W., Chen, M., Cai, Z., Zhan, P., Liu, X., Zhu, S., Ye, M., Lv, T., Lv, J., Song, Y., Wang, D., 2024. Efficacy of immunotherapy in patients with oncogene-driven non-small-cell lung cancer: a systematic review and meta-analysis. Therapeutic Advances in Medical Oncology 16. https://doi.org/10.1177/17588359231225036.). Can you find any explanations?
Reply: This might be due to higher frequency of co-mutations and higher TMB. Data trying to explain in the text.
Changes in the text: Lines 296-299 and References added 17-18

Reviewer B
The clinical observation of extremely prolonged PFS in a small number of NSCLC patients harboring BRAF mutations is noteworthy. By and large, BRAF mutant NSCLC patients have little benefit from immune checkpoint inhibitors (ICI) within 2 to 3 months, as was already observed across European countries in the IMUNOTARGET registry study (reference 15 in the manuscript). Therefore, the points to be addressed are:

1. Could you match the characteristics of these selected sub-group with those of often BRAF mutant NSCLC non-responders to ICI?

Reply: This might be due different class of mutation, as well as higher frequency of co-mutations and higher TMB. Data trying to explain in the text.
Changes : lAdded ines 292-295 and References added 18-19

2. Since NGS, as stressed, is of great usefulness and deserves to be used, what about co-mutations that this group of BRAF patients with such favorable PFS were carrying out? What about TP53 status?
Reply : TP53 was only assessed in one patient , we have no more data to draw any conclusions about it.
Changes : no changes

3. In melanoma, BRAF mutant patients achieve long-term outcomes with immunotherapy, and also the sequence of treatments matters, with more favorable

clinical outcomes with upfront ICI followed by targeted therapy, which is certainly a paradox in contrast with BRAF mutant NSCLC. See the melanoma references in Wolchok et al. J Clin Oncol 2021, Ascierto et al. J Clin Oncol 2022, Atkins et al. J Clin Oncol 2022, Ascierto et al. Lancet Oncology 2023. The clinical findings reported in this small group of BRAF mutant NSCLC patients treated with ICI are similar to the overall results attained with ICI in the overall population of BRAF mutant melanoma patients. Hence, a major understanding of the reasons for this unexpected finding of such long PFS in this small, identified group of BRAF mutant NSCLC patients warrants in-depth explanation.
Reply: Your valuable conclusion was added in the text as well as the newest reference.
Changes : Added line 308-315 and ref 21

4. Commonly, NSCLC patients are refractory to ICI because they have minimal expression of MHC-I by multiple alterations in the cGAMP-GAS-STING signaling pathway that should be kept in consideration in the study presented.
Reply : There is data added in the manuscript according to your suggestion.
Changes : Added : Line 231-233