Peer Review File

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**1st Round**

**Reviewer A**

**Comment 1:** I reviewed the paper and honestly, I think this is very good.
**Reply 1:** thank you for reviewing the manuscript and commenting on this.

**Reviewer B**

**Comment 1:** The review provides in general a good overview over the NTRK field at present, specifically related to lung cancer. The title gives impression of a discussion on what therapy to select as first-line treatment in NTRK-positive cancer, but this is not mentioned at all. Perhaps either change title, or incorporate some speculations/extrapolations from other driver mutations that targeted therapy should be selected before immuno(chemo)therapy? Furthermore – the last part of the title "Focus on NTRK" seems a bit odd as everything in this paper is about NTRK.
**Reply 1:** thank you for this valuable comment. To clarify this, the following text has been added to the Discussion part: "There is still scarce data about the optimal treatment sequence of TRK inhibitors in relation to other therapies, including chemotherapy and immune checkpoint inhibitors, and also the efficacy of these other treatments in *NTRK* fusion-positive NSCLC. In parallel, there is data indicating that immune checkpoint inhibitors in other oncogenic-driven lung tumors, including Epidermal Growth Factor Receptor (EGFR)-mutated and ALK-rearranged NSCLC have limited efficacy. Considering the high response rates of TRK inhibitors also in first line, and the scarcity of data for other therapies, it can be recommended that TRK inhibitors are used as first-line therapy if the *NTRK* fusion is discovered prior to the start of first-line systemic therapy. Upon progression, initial systemic therapy options used for other non-oncogenic- driven NSCLC can applied or alternatively inclusion into clinical trials with next generation TRK-inhibitors. If the *NTRK* fusion is discovered during first-line systemic therapy there is an option to complete the ongoing systemic therapy or interrupt the ongoing treatment and switch to a TRK-inhibitor depending on the clinical situation. These recommendations are also in line with the FDA and EMA approvals and the NCCN (National Comprehensive Cancer Network) guidelines."
Furthermore, the title has been modified to better accommodate the different lines of treatments available for *NTRK* fusion-positive NSCLC. The modified title is now "How Selecting Best Therapy for Metastatic *NTRK* Fusion-Positive Non-Small Cell Lung Cancer?"

**Comment 2:** In the mentioning of NTRK in normal tissues (page 3 in my version, no line numbers), perhaps a notion on NTRK protein expression in normal lung tissue could be of interest – to inform the use of IHC.
**Reply 2:** on page 4, line 102-105, under "*NTRK* genes and TRK receptors" the following text has been added to clarify the protein expression of neurotrophins and their receptors in normal lung tissue: "In human lung tissue, neurotrophins and their receptors have been showed to be expressed on a protein level in different components of

the lung, including bronchial epithelial cells and alveolar cells, suggesting a role in the regulation of normal lung function(10)."

**Comment 3:** Second line page 5: mouse is misspelled as mosue, and upper part page 6, ETV6 is misspelled as EVT6.
**Reply 3:** thank you for pointing out these misspellings. On page 5 second line, the correct spelling of mouse has now been inserted. On page 6, the correct spelling of ETV6 has been inserted.

**Comment 4:** Page 7: Missing 'is': "IHC has several advantages: it is used commonly…"
**Reply 4:** "is" has now been inserted on page 7 line 188: "it is commonly used…."

**Comment 5:** Same page: a reference for the IHC sensitivity/specificity data is missing. And is IHC really sufficient for treatment-decisions, are there data on IHC-only positivity and NTRK-TKI responses (isn't "recommended" to weak)?
**Reply 5:** two references have been inserted for the sensitivity and specificity of IHC: reference number 26 and 27.
To stress the need for confirmation of IHC findings with other nucleic acid-based techniques, the wording has been changed to "needed" instead of "recommended" according to: "….but confirming positive IHC stainings with nucleic acid-based testing is needed."
Also, another sentence has been inserted directly after the one mentioned above to point out the need for IHC confirmation of *NTRK* fusions: "On the other hand, IHC should be used to confirm protein expression of *NTRK* fusions detected by nucleic acid-based testing since not all *NTRK* fusion genes are expressed and the protein kinase is the pharmacological target(27)."

**Comment 6:** Page 8-11: The mentioning of clinical trial results for both larotrectinib and entrectinib seems a bit repetitive, due to several studies reporting at different time points on the same patient material. Perhaps sum up based on the latest publication.
**Reply 6:** for larotrectinib**,** on page 9 lines 217-223, the text has been shortened to avoid repetition of updated reports and only the latest publication has been included. The text has been shortened to the following: "There have been several reports from these studies. Responses have been reported to occur regardless of tumor type and treatment considered tolerable with the majority of adverse events (93%) being grade 1 or 2. The most common adverse events included anemia (11%), increased liver enzymes (7%), weight increase (7%), and decrease in neutrophil count (7%). Other adverse events reported include fatigue, dizziness, nausea and constipation. Recently, a pooled analysis of the three trials was published with 159 TRK fusion-positive cancer patients(32)."
For entrectinib, on page 10-11 (lines 272-312) the text has been shortened for the report from Demetri and colleagues and expanded for the latest updated publication by Doebele et al., including safety analyses. Please refer to manuscript with track changes to see the modifications.

**Comment 7:** Page 11: I assume development of resistance is unavoidable also in NTRK-treated patients, so perhaps "…resistance to treatment can occur" is a bit understating?

**Reply 7:** thank you for pointing this out. The wording on page 11 lines 322-323 has been changed to stress the major problem of acquired resistance, still recognizing the long lasting ongoing responses to TRK inhibitors reported: "despite high clinical activity of TRK inhibitors, acquired resistance to treatment is a major clinical issue and cases of resistance to entrectinib and larotrectinib have been reported.

**Comment 8:** Page 13, upper part: perhaps move "in the low nanomolar…" to between "…in vitro activity" and "against wild-type…"
Same page few lines below: double with. Same line: according to abstract, only 25 of the patients were evaluable (4 were non-evaluable).
**Reply 8:** on page 13 lines 364-365 "in the low nanomolar range" has been moved as suggested: "Both these inhibitors have demonstrated *in vitro* activity in the low nanomolar concentration range against both wild-type TRKA/B/C and many of the resistant TRK mutants."
Page 13 line 369, the double "with" has been removed.
Page 13 lines 369-371: the text has been modified to recognize the 4 non-evaluable patients: "25 of the total 29 patients were evaluable for response and ten of the patients (34%) had a confirmed complete or partial response."

**Comment 9:** Page 14, 2nd line: 'paediatric' is elsewhere termed 'pediatric'
**Reply 9:** the term "pediatric" has been inserted on page 14, 2nd line.

**Comment 10:** There is a figure, but there is no reference to this illustration in the text as far as I can see. I wonder if the lay-out of the figure could be optimized. The tables too lack reference in text.
**Reply 10:** on page 4 line 110, a reference to Figure 1 has been added: "Generally, the fusion eliminates the ligand binding site, resulting in ligand-independent dimerisation and phosphorylation of the receptors (Figure 1)." Also, on page 11 line 323 another reference to Figure 1 has been inserted: "This resistance can be mediated by on-target and off-target mechanisms (Figure 1)."

Figure 1 has been modified to optimize the lay-out, please see new version of Figure 1. Figure legend to Figure 1 on page 18, lines 564-565, has also been modified with addition of abbreviations.

On page 9, line 216 (first line), a reference to Table 1 has been added for larotrectinib: "….adults (NAVIGATE)(Table 1)".
On page 10, line 271 , a reference to Table 1 has been added for entrectinib: "…..I/Ib pediatric trial (STARTRK-NG)(Table 1)."
On page 13 , line 361 , a reference to Table 1 has been added for the next generation TRK inhibitors: "….second generation TRK inhibitors in clinical development (Table 1)…"

**2nd Round**

**Comment 1:** There are still some typos in the text to be edited.
**Response:** typos have been corrected.

**Comment 2:** Table 1: 95% confidence intervals should be provided for both median PFS and OS. Toxicity data in terms of grade 3-5 adverse events could be also reported as a separate column.
**Response:** 95% confidence intervals for PFS and OS have been inserted in Table 1 where available data. Toxicity data as grade 3-5 AEs have been added in a separate column in Table 1.

**Comment 3:** Table 2: "Kinase inhib" editing required.
**Response:** In Table 2, ”Kinase inhibitor” has been spelled out.

**3rd Round**

**Comment 1:** Table 1 should include only published trials with activity or toxicity data. Please limit to cite trials without any data available/published within the text. Furthermore it's not clear why toxicity data have not been reported for drilon', hassen', and hyman's studies. If there are not grade 3-5 trAEs for these studies it should be reported as 0%. otherwise "not available" or "not reported" should be included.
**Response:** I just want to confirm that we mean Table 2 and not Table 1. One ongoing study with selitrectinib (NCT 03215511) and one ongoing with repotrectinib (NCT 03093116) have been removed from Table 2 because of lack of available/published data. In Table 2, trAEs gr. 3-5 have been added for the studies by Drilon and Lassen, for Hyman NA has been added (not available). For the other studies, % for each grade of trAEs has been added, even if 0%.