Peer Review File

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Reviewer A:

Comment 1: Line 33: please consider to change "a leading cause" in "the leading cause".

Reply 1: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: Lung cancer is the leading cause of cancer-related deaths worldwide (1).

Comment 2: Line 35: metastases instead of metastasis.

Reply 2: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: SCLC accounts for approximately 15% of all lung cancers and is characterized by a high proliferation rate, strong predisposition for early metastases, and poor prognosis.

Comment 3: Line 39: please modify as: "showing that 60–68% of patients achieved an objective response"

Reply 3: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: Recently, notable advancements have been made in combining a cytotoxic agent and an immune checkpoint inhibitor as a first-line treatment for extended-stage (ES-) SCLC, showing that 60–68% of patients achieved an objective response (2, 3).

Comment 4: Lines 41-45: please modify all the sentence as: "NSCLC therapeutic landscape have been enriched by remarkable achievements especially for targeted therapies in case of gene alterations. Unfortunately, such advancements have not been observed in SCLC, despite significant efforts in this direction."

Reply 4: Thank you for your suggestion. As advised, we have revised our manuscript. We have made minimal grammatical corrections.

Changes in the text: NSCLC therapeutic landscape has been enriched by remarkable achievements especially for targeted therapies in case of gene alterations. Unfortunately, such advancements have not been observed in SCLC, despite significant efforts in this direction.

Comment 5: Line 53: please mention the study also in the text: author 1 et al. published in XX journal last XX.

Reply 5: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: In this editorial commentary, we consolidate the surrounding issues regarding the therapeutic approaches and utility of predictive biomarkers, based on a study (5) that elucidates the role of BCL-2 expression as a new biomarker for AURKB inhibition in SCLC (K. Ramkumar et al., published in August 2023).

Comment 6: Lines 75-84: please modify by including clinical trial ID number. Also this part should be more detailed and informative. An update should be provided. Consider to include "Park S, Shim J, Mortimer PGS, Smith SA, Godin RE, Hollingsworth SJ, Kim HJ, Jung HA, Sun JM, Park WY, Ahn JS, Ahn MJ, Lee SH, Park K. Biomarker-driven phase 2 umbrella trial study for patients with recurrent small cell lung cancer failing platinum-based chemotherapy. Cancer. 2020 Sep 1;126(17):4002-4012. doi: 10.1002/cncr.33048. Epub 2020 Jun 25. PMID: 32584426."

From clinicaltrial.gov -> NCT04525391: AZD2811 and Durvalumab (MEDI4736) Combination Therapy in Relapsed Small Cell Lung Cancer (SUKSES-N5).

Please correct the table.

NCT04745689 -> AZD2811 and Durvalumab Combination as Maintenance Therapy. This should be specified, both in the text and in the table as the context is completely different and readers may esitate in wrong conclusions.

Reply 6: Thank you for your suggestion. We appreciate the reviewer's insightful comments and have undertaken a comprehensive revision of both the manuscript and Table 1. Our revisions include:

Clarifying and correcting instances of trial confusion within the table and the manuscript.

Enhancing the clarity of the manuscript by including both NCT numbers and more recognizable abbreviations or IDs for each trial, with a commitment to using these abbreviations consistently throughout the text.

Introducing a new column to detail the line of treatment.

Ensuring that trials under specific and unique circumstances are discussed separately in the main text to avoid confusion.

Rectifying the mislabeling of 'chiauranib' as 'chiauratinib' throughout the document.

We apologize for the oversight and assure the reviewer that the necessary corrections have been made to address the concerns raised.

Changes in the text: AZD2811 is a selective AURKB inhibitor that has been investigated in three phase II trials; NCT03366675 (SUKSES-N3) and NCT04525391 (SUKSES-N5) are part of a multi-arm phase II trial examining second or third-line treatments for recurrent SCLC patients, allocated as biomarker non-selected arms (13). In SUKSES-N3, which evaluated the single-agent AZD2811, 15 patients were enrolled. This trial showed limited clinical efficacy of the drug as a monotherapy, with no objective response and a median progression-free survival of 1.6 months (95% CI: 0.9-1.7 months). SUKSES-N5, examining the combination of AZD2811 and the anti-PD-L1 antibody durvalumab, had four patients allocated but was recommended for termination owing to suspected unexpected serious adverse reactions (SUSAR) in another trial using the same drugs. NCT04745689 (TAZMAN) is being conducted with a regimen similar to SUKSES-N5. It is a single-arm phase II trial that evaluates the safety and efficacy of combining AZD2811 with the standard maintenance therapy of durvalumab. This trial targets patients who did not progress after induction therapy with platinum + etoposide + durvalumab, which is one of the current standard first-line treatments. To date, nine patients have received the combination therapy of durvalumab and AZD2811, and the results have not yet been posted yet. It is crucial to note that the latter two trials (SUKSES-N5 and TAZMAN) are combination studies with immune checkpoint inhibitors, not monotherapy

trials of AZD2811. Furthermore, it is important to note that the last trial examines combination therapy in first-line treatment; this differs from the other trials, which focus on recurrent SCLC. These distinctions in the treatment setting should be carefully considered. Chiauranib, or CS2164, is a potent multi-kinase inhibitor of AURKB, VEGFRs, and colony-stimulating factor-1 receptor (14). Tree trials are currently investigating chiauranib; NCT03216343 (CAR105) and NCT05271292 (CAR107) are single-group trials administering chiauranib monotherapy to patients with recurrent SCLC. CAR105 is a phase Ib/II trial examining the safety and efficacy of a regimen involving a daily oral dose of 50 mg chiauranib capsules. In the phase II part, 28 patients were enrolled. Of these, 17.9% (95% CI: 6.1%–36.9%) achieved an objective response and the median progression-free survival was 3.6 months. The regimen was well tolerated, although grade 3-4 adverse events (AEs) including hypertension (25%) and hyponatremia (14%) were observed (15). CAR107 consists of a phase Ib part to determine the optimal dose of chiauranib capsules for solid tumors (between 35 and 65mg/day), and a phase II to assess the safety and efficacy of the determined dose in recurrent SCLC. Results, including patient enrollment numbers, have not yet been published. Planned in response to promising results suggested in CAR105, NCT04830813 (CAR302) is a randomized, double-blind, placebo-controlled, multi-center phase III clinical trial to verify the effect of chiauranib monotherapy in patients with recurrent SCLC. It is currently in the recruiting stage. Among the aforementioned trials, it is noteworthy that only CAR302 is designed as a placebo-controlled comparative study, in contrast to the others which are designed as single-arm trials.

Comment 7: Line 85: modify as: (frequently referred to as “AZD2811”).

Reply 7: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: AZD1152 and its improved product, AZD2811NP (frequently referred to as “AZD2811”), are the most widely investigated AURKB inhibitors in clinical trials conducted on malignancies (13).

Comment 8: Line 94: modify as: which shows almost no solubility in water thus not suitable for clinical application.

Reply 8: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: During development, AZD1152 was known as a prodrug and quickly converted to the active drug AZD2811 (previously known as AZD1152-hQPA) in plasma, which shows almost no solubility in water thus not suitable for clinical application.

Comment 9: Line 98: modify as: and may be considered as an AURKB inhibitor, suitable for clinical study (15, 16).

Reply 9: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: However, AZD2811NP, a recently developed nanoparticle-encapsulated AZD2811 with extended drug release and a favorable toxicity-efficacy profile in preclinical models, has been developed and may be considered as an AURKB inhibitor, suitable for clinical study (15, 16).

Comment 10: Lines 99-104: please consider to explicit this concept in a more detailed, informative and clear way.

Reply 10: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: To refine the clinical application of AURKB inhibitors, biomarkers that predict the efficacy of AURKB inhibition are needed to ensure that treatment is given to patients who are predicted to respond, thereby maximizing efficacy and minimizing exposure to unnecessary toxicity. AURKB activity is known to be enhanced by the oncogene c-MYC, which also benefits from AURKB by helping to stabilize the c-MYC protein (17, 18). This interdependence suggests that cancers with high levels of c-MYC might be particularly sensitive to AURKB inhibitors. In support of this, research using AZD1152, an AURKB inhibitor, showed that tumors with c-MYC amplification were more likely to respond to treatment, as evidenced by reduced tumor growth in animal models of SCLC (19).

Comment 11: Lines 117-118: please modify as: interestingly in this study, high BCL-2 expression levels have been correlated to AURKB inhibitors lower efficacy.

Reply 11: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: Interestingly in this study, preclinical models using cell lines and xenografts showed that high BCL-2 expression levels have been correlated to AURKB inhibitors lower efficacy.

Comment 12: Line 120-125: please consider to explicit this concept in a more detailed, informative and clear way.

Reply 12: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text:

Although AURKB and c-MYC have a close mechanistic relationship, leading to the original hypothesis of the utility of c-MYC as a therapeutic biomarker for AURKB inhibition, the expected correlation between high c-MYC expression (estimated from proteomic expression profiling and genomic amplification) and high AURKB inhibitor sensitivity was not observed in the cell line study. Contrary to expectations, it was low BCL-2 expression that emerged as a predictive marker for treatment efficacy. In this study, proteomic expression profiling was performed to examine the levels of BCL-2 family proteins within the cell lines. It was found that cell lines with low levels of BCL-2 protein expression were more sensitive to AURKB inhibitors.

Comment 13: Line 149: please modify as: As already mentioned.

Reply 13: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: As already mentioned, combined chemo-immunotherapy is the standard of care in the first-line treatment of ES-SCLC, and a phase II study is considering the combination of AZD2811NP and immunotherapy.

Comment 14: Line 152: toxicities instead of toxicity.

Reply 14: Thank you for your suggestion. As advised, we have revised our manuscript.

Changes in the text: As combination therapies could be considered actively, it is essential to explore administration methods that balance efficacy and reduce toxicities.

Comment 15: Regarding table 1, please specify the line of treatment for the clinical trials.

Reply 15: Thank you for your suggestion. As advised, we have updated the table to include a column that details the line of treatment. (also mentioned in the reply 6)

Reviewer B

Comment 1: Given the role of Aurora Kinase A in mitosis regulation, there have been a few dozen clinical trials for Aurora Kinase A inhibitors across various cancer treatments. An selective Aurora A kinase inhibitor, LY3295668 (NCT03898791) is currently undergoing clinical trials for SCLC and should be included and discussed in the manuscript, especially given its affinity for Aurora B (with Ki values of 0.8 nM and 1038 nM for AURKA and AURKB, respectively).

Reply 1: Thank you for your valuable suggestion. While we appreciate your suggestion regarding the inclusion of LY3295668, a relatively non-selective Aurora kinase family inhibitor, in our manuscript, we believe it falls outside the scope of our current commentary, which focuses on Aurora kinase B inhibitors. Including Aurora kinase A inhibitors, which are more numerous in clinical trials, might shift the theme of our manuscript. As we mention in our reply to your comment 2, there is preclinical evidence suggesting the theoretical superiority of Aurora kinase B inhibition over Aurora kinase A inhibition. Therefore, we consider it more appropriate not to include the trial you mentioned in our discussion.

Comment 2: An essential aspect to consider is whether a selective Aurora B kinase inhibitor holds superiority over a pan Aurora kinase inhibitor in SCLC treatment. The manuscript should explore and analyze this comparison, providing insights into the potential advantages and disadvantages of each approach.

Reply 2: Thank you for your suggestion. As you suggested, we have added sentences demonstrating the superiority of Aurora kinase B inhibition over pan-Aurora and Aurora kinase A/C. To simplify the structure and clarify the content, we have decided to remove the sentences on the Aurora kinase A inhibition trial in SCLC (which uses c-Myc as a biomarker and provides implications for our theme but could cause confusion).

Due to this modification and the need to address other reviewers' comments, we have decided to change the structure of the manuscript and created a separate paragraph combining the potential of AURKB inhibition in SCLC and the associated clinical trials.

Changes in the text:

AURKs are vital cell cycle regulators; AURKA and AURKB are crucial for mitosis, while AURKC predominantly affects gametogenesis. AURKA and AURKB are widely overexpressed in numerous malignancies (7), and inhibiting them is a key focus of many clinical trials. However, based on preclinical gene knockout studies, selectively inhibiting AURKB may offer distinct advantages. The functionality of AURKB when knocked out could potentially be compensated for by AURKC in the early embryonic phases [a], unlike AURKA, which is indispensable for normal development [b]. Therefore, targeting AURKB inhibition emerges as a promising approach for cancer therapy.

Comment 3: Furthermore, an ongoing clinical trial for chiauratinib (NCT05271292) in relapsed/refractory SCLC adds another dimension to the therapeutic landscape. Table 1 should be updated to incorporate relevant details about this trial, and the findings should be discussed within the manuscript.

Reply 3: Thank you for your suggestion. As advised, we have updated the table to include the ongoing trial for chiauranib (NCT05271292). Moreover, we apologize for the error in our table where 'chiauranib' was mistakenly written as 'chiauratnib.' We have corrected this mistake.

Reviewer C

Comment 1: Line 68: I think it is important to note that biomarker analyses here were exploratory in nature.

Reply 1: Thank you for your suggestion. Indeed, the exploratory nature of the analysis regarding the utility of c-Myc as a biomarker in Aurora Kinase A (AURKA) inhibition, as mentioned in the cited paper, is a very important point. However, in responding to other reviewers' comments, we decided that removing this section and replacing it with a different text would more clearly articulate the argument.

Comment 2: Line 117: Please note that this is in preclinical studies, lest this sentence be taken out of context.

Reply 2: Thank you for pointing that out. Indeed, it was a part that could be misunderstood. We have revised the text to clearly indicate that this refers to preclinical studies.

Changes in the text: Interestingly in this study, preclinical models using cell lines and xenografts showed that high BCL-2 expression levels have been correlated to AURKB inhibitors lower efficacy.

Line 155: Please soften the language and use "potential biomarker" in this paragraph, as we have yet to see the value in human studies.

Comment 4: Table 1: Please add NCT01118611 and NCT00424632

Reply 4: Thank you for your valuable suggestion. In this table, we are focusing on drugs that are 'specific to Aurora Kinase B'. There are many drugs that act non-specifically on Aurora Kinases (for example, GSK1070916A [against Aurora Kinase A/B/C] and PF-03814735 [against Aurora Kinase A/B] as mentioned in the trial you pointed out), and it is not feasible to list them all in this table. To make this clear, we have decided to use the term “selective aurora kinase B inhibitor” in the main text and in the title of the table. Furthermore, we have decided to include in the text the advantages of Aurora Kinase B inhibition over pan-Aurora Kinase inhibition, as well as the numerous trials involving non-specific Aurora Kinase inhibitors.