Peer review file

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Reviewer A

This paper is a comprehensive overview of ongoing clinical trials and therapeutic approaches to treating small-cell lung cancer. The subheadings are easy to follow. While the authors cover many relevant topics and the summary tables are useful, the main weakness of this review is that it neither stimulates a new view or innovative idea for further investigation for the field of SCLC. The authors should provide their perspective of the clinical outcome results and insight on how discordant or promising results should be interpreted and impact the current standard of care for SCLC. The review would benefit from improving the following issues:

Comment 1: Perhaps one of the most anticipated clinical trials for SCLC in recent times has been the mixed results from the KEYNOTE-604 trial (Phase III pembrolizumab + chemotherapy), which is listed in the supplementary table but not discussed in depth in the text. Therefore, it leaves one wondering whether the authors have curated the most relevant clinical trials pertaining to SCLC.

Reply 1: Thank you for your valuable feedback. We added the detailed illustration of this recent study in this version, and adjusted the text where highlighted (see Page 17, Line 320).

Changes in the text:

Recently, MK-3475-604/ KEYNOTE-604 (NCT03066778; phase III) has shown mixed results in ES-SCLC that pembrolizumab plus etoposide/platinum could potentially prolong PFS (P = 0.0023; median 4.5 vs 4.3 months) but OS (P = 0.0164; median 10.8 vs 9.7 months)(52).

Comment 2: For all on-going clinical trials without outcome, the authors should describe what data supported the target rationale and potential mechanisms of action in SCLC. The tables are sufficient for listing these studies without results. It is not enough to simply list this in text - more thought and perspective should be placed here.

Reply 2: We are extremely grateful to you for pointing out this problem. To make up for the researches of some combinations have not yet obtained clear results, we tried to supplement some mechanisms why these therapies can be combined with immunotherapy, such as chemotherapy and(or) radiotherapy (Page 15, Line 293), antiangiogenic agents (Page 21, Line 397), Notch pathway inhibitors (Page 26, Line 506), KRAS pathway inhibitors (Page 27, Line 526), L1S1 inhibitors (Page 28, Line 541), and LXR agonist (Page 29, Line 556). All of these adjustments were highlighted in the text.

Changes in the text:

(1) Chemotherapy and radiotherapy are beneficial to mature antigen-presenting cells, increase PD-L1 expression on tumor cells, decrease tumor-infiltrating Tregs or MDSCs, and recruit CD8+ TILs and macrophages (5). These shaping of TME from chemotherapy and radiotherapy make them become the first choice in combination with immunotherapy.

(2) A series of mice models demonstrated that the antiangiogenic therapy targeting vascular endothelial growth factor (VEGF) and Angiopoietin-2 (ANG2)(61), and vascular endothelial growth factor receptor 2 (VEGFR2)(62) raised the level of PD-L1 on endothelial cells (ECs) and tumor cells, leading to the suppression of antitumor immunity. Thus, a piece of equitable evidence supports the combination of antiangiogenic agents and ICIs, to solve the resistance during the antiangiogenic therapy.

(3) A study of colorectal carcinoma revealed the blockade of Notch pathway could stimulate tumor-infiltrating CD8+ cytotoxic T lymphocytes (CTL), and the production of IFN-γ, TNF-α, IL-1β, IL-6, and IL-8(80). What is more, the PD1 expression on CD8+ CTL was also declined with the inhabitation of Notch pathway, demonstrating the great potential to assist PD-1 blockers.

(4) Although there has not been a clear conclusion in SCLC, several surprising ICIs in KRAS mutant NSCLC have been reported. According to a meta-analysis, ICIs

prolonged the OS of KRAS mutant NSCLC, compared with chemotherapy (P = 0.03)(84). Therefore, whether KRAS mutation could also be a potential biomarker for the efficacy of ICIs in SCLC is worth discussion.

(5) Mounting results in breast mice models showed that the combination of LSD1 inhibitors with PD-1 antibody significantly inhibited tumor growth, decreased Ki-67 level, and enhanced CD8+ TILs(87). Overall, these results proved that LSD1 inhibition could be an approach to assist with immunotherapy.

(6) RGX-104, an oral administered LXR agonist, was proved to upgrade LXR-mediated expression of apolipoprotein E (ApoE), which induced apoptosis of peripheral or tumor-infiltrating MDSCs, and then supported T cell-engaging antitumor immunity(91). Consequently, this therapy was considered to promote the curative effect of ICIs.

Comment 3: For CDK inhibitors, a more recent preclinical paper, Zhang H. et al, 2020, has shown potential efficacy of CDK7i in SCLCs. Is CDK4/6 the only CDKs considered for SCLC?

Reply 3: Thank you for your insightful reminder. To ensure the comprehensiveness of this article, we added the recent evidence from Zhang H. et, al.'s research (see Page 23, Line 444).

Changes in the text:
Additionally, a selective CDK7 inhibitor, YKL-5-124, was another potent treatment in SCLC mice models in Hua Zhang et al.'s study (69). Anti-PD-1 + YKL-5-124 presented with a better response than a single treatment. Strikingly, the mice treated with four-drug combination (YKL-5-124 + anti-PD-1+EC) appeared to live significantly longer than those treated with anti-PD-1+EC or EC alone. Moreover, this study also indicated that YKL-5-124 induced antitumor immune reactions engaging DCs (MHCII+CD11c+CD103+), effector CD4+T cells (CD44highCD62Llow/ Ki67+/ ICOS+) and cytotoxic CD8+T cells (Granzyme B+), which could be heightened by the combination with PD-1 blockades.

Comment 4: The future perspective section would benefit from more structure. EGFR mutations are rare in SCLC and is not mentioned elsewhere in the text, so it's not clear why it is mentioned here for the first time. Reference 84 is also incorrectly cited. Please check all citations for accuracy. This section should be a summary of the most relevant topics discussed above.

Reply 4: Thank you for your careful reading. According to your advice, we added some new perspectives and separated the future perspective section into several subheadings, including "Development of more predictive biomarkers" (Page 30, Line 573), "Specific clinical trial designs" (Page 22, Line 569), "Enhancement of the efficacy" (Page 30, Line 586), and "Decreasing the financial toxicity" (Page 31, Line 597). All of these adjustments were highlighted in the text. Moreover, the content mentioned EGFR and reference 84 has been deleted in this version.

Changes in the text:

1 Development of more predictive biomarkers

First, lacking potential biomarkers to predict the therapeutic effect of ICIs alone or in combination with other therapies is concerned in SCLC(92). High level of PD-L1 in multiple tumor cells or TME had been assumed as a predictor for a better response and survival after ICIs treatment, which has been approved by the FDA as an indicator for variable solid tumors, including lung cancers. However, recent evidence suggested that the clinical benefit of ICIs might not be restricted in PD-L1 high patients, leading to the full doubt of the predictive capability of PD-L1(93). Thereby, multimodal detection of PD-L1, such as PD-L1 protein, PD-L1 mRNA, circulating PD-L1, and dynamic PD-L1 monitoring, might provide a comprehensive method for screening candidates for ICIs. Moreover, combined biomarkers, such as PD-L1 combined with tumor mutation burden (TMB) and microsatellite instability-high/ mismatch repair deficient, nomograms, and deep learning, could better provide advice for therapeutic decisions (94).

2 Specific clinical trial designs

10–25% of patients beneficial from ICIs are not counted by the traditional response criteria, due to the similar imaging performance of inflammation and tumor growth, the antitumor activity of immunotherapy might be more substantial than measured by

standard response criteria (95). Secondly, due to the immunotherapy is based on slow stimulation of the immune system rather than rapidly assaulting tumor cells, the efficacy and antitumor responses are always delayed (96). Thereby, the endpoints of traditional anticancer treatment may be insufficient for immunotherapy, and long-term disease-free survival is more appropriate for immunotherapy (97). Moreover, the assessment of multiple immune cells in the TME or peripheral blood should be counted as additional endpoints for immunotherapy (97).

3 Enhancement of the efficacy

Tumor heterogeneity and treatment resistance are the most two mechanisms to hinder the efficacy of cancer immunotherapy (93). Liquid biopsies, containing the genomic analysis of circulating free DNA or cancer cells, could noninvasively obtain the level of tumor heterogeneity and also dynamically monitor resistance (98). Moreover, liquid biopsies are promising for personalized medicine to screen optimal combinational therapy (98).

4 Decreasing the financial toxicity

Cancer immunotherapies are known as one of the most promising treatments for malignant tumors, while they are also the most expensive ones in cancer, considering the enormous research costs (99). The cost for each lung cancer patient to treat with nivolumab was estimated at $44,100 per year (100). Thence, screening potential patients for immunotherapy is critical to reducing the unnecessary financial burden on patients. Meanwhile, cost-effectiveness, cost-benefit, and quality of life (QoL) is another approach to weigh the clinical benefit and financial cost (93).

Comment 5: Supplemental table 1 should be an in-text table 3.

Reply 5: Thank you for your comment. We put the previous supplementary Table 1 into the main body as new Table 3 in this version (see Page 62).

Comment 6: The paper should be edited for grammar and writing.

Reply 6: Thank you for your careful reading. We are sorry for our previous spelling and grammar mistakes in the text. As for this version, we checked and modified the text via an automated English checker named Grammarly. And we hope this version could meet

your expectation.

Reviewer B

In this review, the authors offer a general view of combinational immunothrapy of SCLC. SCLC is a kind of highly aggressive cancer. Over 30 years, the treatment of SCLC has no progress. Until 2018, EP plus ateolizumab was approved by the FDA. So this review is of good value because this field is moving forward very fast.

This manuscript is divided into 2 parts, the basic knowledge part and the clinical part. The clinical part is organised well and well written. However, there are some shortcomings in the basic knowledge part. These shortcomings should carefully be corrected.

Comment 1: In line 77, the author claims that "CTLA-4 is a surface or intracellular molecule on effector T cells". I think activated T cells is proper here because Treg cell also express CTLA-4 at a high level.

Reply 1: Thank you for your careful reading. In this version, we replaced activated to effector in Page 5, Line 90.

Changes in the text:

CTLA-4 (CD152) is a surface or intracellular molecule on activated T cells that curbs the initial period of T cells activation as a competitive ligand for the T-cell costimulatory receptor CD28(6).

Comment 2: In line 80, the author claims that "...is transported to the surface upon activated by binding CD28". CTLA4 transportation to immune-synapse is controlled by TCR signaling, but not CD28.

Reply 2: Thank you for your insightful comment. In this version, we replaced T cell receptor (TCR) to binding CD28 in Page 5, Line 93.

Changes in the text:

CTLA-4 is mostly found in intracellular compartments and is transported to the surface upon activated by T cell receptor (TCR) (7).

Comment 3: Mounting evidence suggests that ipilimumab exerts its function by depleting Treg, which should be included in this section.

Reply 3: We are grateful for you to point out this problem. We supplemented the information of the process of Treg depletion in Page 5, Line 94, and Page 5, Line 96.

Changes in the text:
Additionally, CTLA-4 can both regulate CD4+ T cells and selective depletion of Tregs by removing CD80 and CD86 from the cell surfaces of antigen-presenting cells (APCs) via trans-endocytosis(8-10) (Figure 1). Further, a recent study developed a dual variable domain immunoglobulin of anti-CTLA4 antibody, which could deplete intratumor Tregs, but spares tissue-resident Tregs, minimizing potential toxicities of CTLA-4 blockades(11).

Comment 4: In line 97, the author claims that PD-1 is induced by IFN-gama and cited a paper "Atanackovic D, Luetkens T, Kröger N. Coinhibitory molecule PD-1 as a potential target for the 569 immunotherapy of multiple myeloma. Leukemia 2014;28:993-100". However, I carefully read this paper, but I found no evidence suggest PD-1 is induced IFN-gama. To my knowledge, PD-1 is mainly induced by TCR signaling and some cytokines including IL-2, IL15 could up-regulate PD-1.

Reply 4: We are sorry for this writing mistake. Indeed, we wanted to express IFN-γ can upgrade the level of PD-L1. Therefore, we corrected it in Page 7, Line 123.

Changes in the text:
Importantly, the cytokines secreted by inflammatory cells, especially interferon-γ (IFN-γ), can induce or maintain the PD-L1 protein expressions(21), showing an IFN-γ/PD-L1 axis between tumor cells and the TME (Figure 2).

Comment 5: Line 99, " ...express PD-L1 and less PD-L2, which correlates with adverse

prognosis" should be revised. Besides, correlation of PD-L1 expression and prognosis of cancer patients is still under debate. Although PD-L1 is an immunosurppressive factor, PD-L1 expression is regulated by IFN-gama, which reflect the activation extent of anti-tumor immunity.

Reply 5: Thank you for your careful reading. In this version, we deleted that sentence and corrected this mistake in Page 7, Line 123.

Changes in the text:
Importantly, the cytokines secreted by inflammatory cells, especially interferon-γ (IFN-γ), can induce or maintain the PD-L1 protein expressions(21), showing an IFN-γ/PD-L1 axis between tumor cells and the TME (Figure 2).

Comment 6: Line 12, the author cited "Gordon SR, Maute RL, Dulken BW, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 2017;545:495-9" to suggest the expression of PD-L1 in TAMs. However, no evidence of PD-L1 expression in TAMs exists in this paper. This raised my concern. I checked some references and found ref14 is cited incorrectly.

Reply 6: Thank you for your valuable feedback. We are sorry for this wrong reference. In this version, we put the correct one (Liu Y, zugazagoitia J, Ahmed FS, et al. Immune cell PD-L1 co-localizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy. Clinical Cancer Research 2019:clincanres.1040.2019.) in Page 6, Line 117.

Changes in the text:
Besides tumor cells, PD-L1 and PD-L2 can also be found on other cells such as macrophages (13), myeloid DCs(14), MDSC(15), stromal fibroblasts(16), and endothelial cells(17). The expression of PD-1 not only inhibits T cells (CD8+) mediated cell killing and promotes the differentiation of exhausted CD8+ T cells (Tex)(18) but also facilitates the differentiation of the Treg (CD4+ Foxp3+) cells from naive CD4+ T cells(19,20).