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Review Article

The progress of PD-1 inhibitors in small-cell lung cancer

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Abstract: Recently, the immune checkpoint inhibitors have shown significant clinical responses in a variety of malignancies, including melanoma, non-small-cell lung cancer (NSCLC) and renal carcinoma etc. Despite numerous clinical trials, the survival of patients with small-cell lung cancer (SCLC) has not changed significantly in the past several decades. And there are some clinical evidence showing involvement of the suppressed immune system in the SCLC development. Therefore, it is worth exploration whether the immune checkpoint blockade for immune enhancement could produce anti-tumor effects as a potential treatment strategy for patients suffering SCLC. The phase III clinical trial, CA184-156 suggested that ipilimumab, the cytolytic T lymphocyte-associated antigen (CTLA-4) inhibitor, combined with platinum-based doublet chemotherapy (PT-DC), could not prolong their life significantly. However, according to some animal experiments, combination blockade of the programmed death-1 (PD-1) and CTLA-4 coinhibitory molecules coupled with Fvax vaccination in the B16 melanoma treatment results in 65% of preimplanted tumors rejection. And on the part of clinical trials, several studies have shown the certain efficacy of PD-1 inhibitors, nivolumab and pembrolizumab in the SCLC treatment. In this review, we summarized the preliminary research findings of the PD-1 inhibitors, nivolumab and pembrolizumab treating SCLC, in order of preclinical studies, phase I, II and III clinical trials.

Keywords: Small-cell lung cancer, PD-1 inhibitors, animal experiments, clinical trials

Introduction

In 2017, there are 1,688,780 new cases of cancer and 600,920 cancer deaths that are projected to occur in the USA, and lung cancer with 222,500 new cases and 155,870 cancer deaths, has become the malignant tumor with the highest incidence and mortality [1]. Small-cell lung cancer (SCLC) accounting for approximately 15% of all lung cancers, is notorious for its rapid doubling time, propensity for metastases and high growth fraction [2, 3]. The 5-year survival rate of SCLC remains low at <7% overall, <5% of patients with extensive-stage SCLC (ES-SCLC) survive for >2 years, and most patients survive for only 1 year or less after diagnosis [4, 5]. Although the tumor cells are highly sensitive to initial chemotherapy, about 80% of patients with limited-stage SCLC (LS-SCLC) and nearly all patients with ES-SCLC eventually relapse and develop progressive diseases [6, 7]. When first-line treatment fails, topotecan is the only approved agent in the United States and Europe for second-line treatment, but it is not widely used in the United States because of disappointing response rates (7-24%) and significant toxicity, such as thrombocytopenia and anemia [8-10]. Therefore, it is urgent to explore the standard second-line treatment for patients who experience progression of disease after first-line chemotherapy.

With the rapid development of immunotherapy in recent years, many scholars wonder whether patients suffering SCLC could benefit from the immune checkpoint inhibitors, which have yielded brilliant fruits in treating melanoma [11], NSCLC [12] and renal carcinoma [13]. There is some clinical evidence supporting involvement of the suppressed immune system in the development of SCLC. (a). Some SCLC patients suffer from paraneoplastic neurologic disorders (PNDs), such as Lambert-Eaton myasthenic

Table 1. Completed Study of Immune Checkpoint Inhibitors in Patients with SCLC

Trial identifier/study name	Agent	Phase	Population (n)	Primary end point	Arms (n)	Results	Ref.
CA184-156/NCT01450761	Ipilimumab	III	Untreated ES-SCLC (1414)	OS	Ipilimumab+EP (478) Vs. Placebo+EP (476)	Median OS (95% CI); months 10.97 (10.45 to 11.33) Vs. 10.94 (10.02 to 11.50) HR, 0.936; (95% CI: 0.807 to 1.085; p=0.3775)	[29]
NCT00527735	Ipilimumab	II	Untreated SCLC (334)	irPFS	Ipilimumab/Placebo + Paclitaxil/Carboplatin (Concurrent, 113) Vs. Ipilimumab + Paclitaxel/ Carboplatin (Sequential, 110) Vs. Placebo + Paclitaxel/Carboplatin (111)	Median irPFS (95% CI); months 5.52 (4.17 to 6.74) Vs. 5.68 (4.76 to 7.79) Vs. 4.63 (4.14 to 5.52) HR (sequential vs. placebo), 0.724; (95% CI: 0.495 to 1.059; p=0.0473*)	[27]
NCT01331525	Ipilimumab	II	Untreated ES-SCLC (42)	1-year PFS	Ipilimumab+EP	15.8% (6/38)	[28]

SCLC: small-cell lung cancer; *ES-SCLC:* extensive-stage SCLC; *EP:* etoposide+cis/carboplatin; *OS:* overall survival; *CI:* confidence interals; *HR:* hazard ratio; *irPFS:* immune-related progression-free survival. *Statistically significant

Table 2. Ongoing and Future Studies of Immune Checkpoint Inhibitors in Patients with SCLC

Trial identifier/ study name	Agent	Phase	Estimated patients enrollment (n)	Arms	Primary end point	Secondary end point	Status
Checkpoint inhibitor combinations (maintenance therapy)							
CheckMate 451/CA209-451/NCT02538666	Ipilimumab nivolumab	III	ES-SCLC after first-line CT (810)	Nivo monotherapy Vs. Nivo+ipi Vs. Placebo	OS, PFS	ORR, Time to treatment failure, toxicity	Active, not recruiting
STIMULI/NCT02046733	Ipilimumab nivolumab	II	LS-SCLC after first-line CT (260)	Nivo+ipi→nivo maintenance Vs. Observation	OS, PFS	PFS descriptive study, OS descriptive study	Recruiting
Nivolumab							
Checkmate 331/NCT02481830	Nivolumab	III	Refractory SCLC (568)	Nivo Vs. Topotecan Vs. Amrubicin	OS	PFS, ORR	Active, not recruiting
NCT03382561	Nivolumab	II	Untreated ES-SCLC (150)	Nivo+EP Vs. EP	PFS	ORR, toxicity, OS	Not yet recruiting
Pembrolizumab							
First-line or maintenance treatment							
KEYNOTE 604/NCT03066778	Pembrolizumab	III	Untreated ES-SCLC (430)	Pembro+EP vs. placebo+EP	PFS, OS	ORR, DoR, toxicity	Recruiting
NCT02580994	Pembrolizumab	II	Untreated ES-SCLC (118)	Pembro+EP vs. EP	PFS	OS	Recruiting
NCT02359019	Pembrolizumab	II	ES-SCLC after first-line CT (54)	Pembro	PFS	OS, Modified PFS*	Active, not recruiting
Second-line treatment							
NCT02963090	Pembrolizumab	II	Refractory SCLC (98)	Pembro vs. Topotecan	PFS		Recruiting
NCT02551432	Pembrolizumab	II	Refractory SCLC (26)	Paclitaxel→paclitaxel+pembro→pembro maintenance	ORR	PFS, OS, Toxicity	Active, not recruiting
NCT03253068	Pembrolizumab	II	Refractory SCLC (25)	Pembro+amrubicin	OS		Not yet recruiting

SCLC: small-cell lung cancer; *ES-SCLC:* extensive-stage SCLC; LS-SCLC: limited-stage SCLC; *nivo:* nivolumab; *ipi:* ipilimumab; *CT:* chemotherapy; *EP:* etoposide+cis/carboplatin; *OS:* overall survival; *PFS:* progression-free survival; *ORR:* objective response rate; *pembro:* pembrolizumab; *DoR:* duration of response. *Modified PFS defined by RECIST as progression that is confirmed by a second scan at least 4 weeks apart.

syndrome (LEMS), because of an immune response targeting antigens shared between the tumor cells and the nervous system, and it is interesting that these patients often have a better prognosis than those without PNDs (median 11.5 months vs. 9.5 months, p=0.095) [14-17]. (b). A study of 64 SCLC biopsy specimens showed that a greater number of CD45+T cells infiltrating SCLC tumors was predictive of better overall survival (OS) despite good performance status (p<0.009) [18]. (c). another study evaluated the peripheral blood samples from 35 consecutive SCLC patients (15 ES-SCLC and 20 LS-SCLC), 8 long-term survivors who have been disease-free for more than 3 years after treatment, and 19 healthy volunteers. Long-term survivors maintained a higher effector T-cell (Teff) to regulatory T-cell (Treg) ratio than patients with recurrent diseases, and significantly more Teff cell numbers were seen in LS-SCLC patients than in ES-SCLC [19, 20]. Above researches all infer that suppressed immune system plays an important role in the development of SCLC, and the immune checkpoint blockade, the brand new cancer treatment strategy, might give SCLC patients substantial clinical benefits.

Ipilimumab is a kind of immune checkpoint, cytolytic T lymphocyte-associated antigen (CTLA-4) inhibitor, approved by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2011 for the treatment of patients with unresectable or metastatic melanoma [21], which augments the anti-tumor immune response by blocking the interaction of CTLA-4 on T cells with its ligands (CD80/CD86) on antigen presenting cells (APCs), and preventing the following down-regulation to Teff. Cytotoxic drugs, such as paclitaxel, platinum, etc, cause the release of tumor-specific antigens, which can be recognized by APCs and combination with ipilimumab for immune enhancement, may provide synergistic effects [22-26]. Many scientists have conducted several clinical trials about ipilimumab combined with chemotherapy for SCLC patients (completed studies as shown in **Table 1** [27-29]). However, according to the phase III trial, CA184-156 [29], phased addition of ipilimumab to etoposide/cisplatin or carboplatin did not prolong neither median OS (11.0 months vs. 10.9 months; hazard ratio [HR], 0.94; 95% confidence interals [CI], 0.81 to 1.09; p=0.3775) nor median WHO-Progression free survival (PFS), which is based on WHO criteria (4.6 months vs. 4.4 months; HR, 0.85; 95% CI, 0.75 to 0.97). As the researches of CTLA-4 inhibitors facing bottle neck, in 2014, another kind of immune checkpoint, PD-1 inhibitors, nivolumab and pembrolizumab came out and attracted attentions from the scholars all over the world. In this review, we summarized the preliminary research findings of these two PD-1 inhibitors in the SCLC treatment, in order of preclinical studies, phase I, II and III clinical trials.

The preclinical trials of immune checkpoint inhibitors

In the normal host, the immune checkpoint serves as a potent negative regulator in the immune system, protects the body tissues from excessive T-cell activation, and plays an indispensable role in induction and maintenance of the immune tolerance, to avoid autoimmune disorders, which is also utilized by many tumors to evade immune surveillance [30]. The best known immune checkpoint is CTLA-4. Ipilimumab is a fully humanized anti-CTLA-4 monoclonal antibody that blocks the binding of CTLA-4 with its ligands, CD80 and CD86 [31], resulting in the continued T-cell activation. Another immune checkpoint is PD-1 receptor, which is expressed on activated T cells, B cells, natural killer cells, monocytes and dendritic cells. When it interacts with its twoligands, PD-L1 and PD-L2, on stromal and tumor cells, PD-1 limits the activity and function of these immune system cells [32-36]. Although CTLA-4 and PD-1 belong to the same family of molecules, some evidence suggests that they use distinct and non-redundant mechanisms to affect T-cell activation. (a). CTLA-4 is expressed on activated T lymphocytes, meanwhile, PD-1 is also expressed on B cells, macrophages [37], dendritic cells [38] and monocytes [39], suggesting involvement in a broader spectrum of immune regulation than CTLA-4. (b). CTLA-4 knockout mice die by 4 weeks of age from a lethal lymphoproliferative disorder [40], whereas some colonies of PD-1 knockout BL6 mice live over a year before manifesting lupus-like symptoms with <50% penetrance [41], inferring that the treatment-related adverse events (TRAEs) of PD-1 inhibitors may be much slighter than blocking CTLA-4. (c). Richard and his colleagues

used DNA microarrays to examine how the differences between CTLA-4 and PD-1 to affect the transcript profile of T cells. The large distinctions between transcripts regulated by CD3/CD28/CTLA-4 and transcripts regulated in the opposite manner, CD3/CD28/PD-1 (3,262 versus 177 transcripts) infers that PD-1 is significantly more potent than CTLA-4, as a suppressor of CD3/CD28-mediated changes in the T-cell transcript profile [42]. (d). The serine/threonine kinase (Akt) is thought to play a crucial role in diverse cellular processes, including cytokine synthesis, survival, and promoting glycolysis [43-48]. Both CTLA-4 and PD-1 inhibit the activity of Akt to affect T cell activation, but through distinct pathways. The intracellular domain of CTLA-4 interacts with the serine/threonine phosphatase, PP2A to increase its activity and then inhibit Akt [49], thus CTLA-4 does not inhibit Akt in the presence of the PP2A inhibitor okadaic acid [42]. On the other hand, PD-1-mediated suppression of Akt activity is completely unaffected by the presence of okadaic acid, because PD-1 abrogates Akt phosphorylation by antagonizing its upstream activator, PI3K, via its intracellular immunoreceptor tyrosine-based switch motif (ITSM) [50, 51]. In conclusion, these two immune checkpoints may produce synergistic effects on tumor progression, and scientists tested this hypothesis by the following two animal experiments.

Currana et al found that combination blockade of the coinhibitory molecules, including PD-1, CTLA-4, and PD-L1, coupled with Fvax vaccination in the B16 melanoma treatment results in 65% of preimplanted tumors rejection and only 10% with CTLA-4 inhibition alone. They also discovered that in the tumor microenvironment, this regimen leads to enhanced Teff infiltration, activation and cytokine production, to the contrary, reduction of negative regulators in the immune system, such as myeloid-derived suppressor cells (MDSCs), Treg and their corresponding activation markers. This experiment warranted the hypothesis that combination blockade PD-1/PD-L1, CTLA-4/B7 and PD-L1/B7-1 interaction can antagonize tumor-induced immune suppression and promote tumor rejection. It is worth mention that blocking a single inhibitory receptor may lead to up-regulation of the other unblocked pathway, getting half the results with twice the effort [52]. Based on the above experiment, Duraiswamy changed the tumor cell lines from B16 melanoma to CT26 colon carcinoma and ID8-VEGF ovarian carcinoma and used Gvax, expressing granulate-macrophage colony-stimulating factor (GM-CSF) to replace Fvax, which expresses Flt3 instead, to further confirm the efficacy of combination coinhibitory receptor blockade in cancer treatment [53]. Similarly, from the tumor rejection rate, Teff/Treg ratios and cytokine production, these two experiments got nearly consistent results. In addition, this preclinical study also performed the adoptive transfer treatment. The CD8+ tumor-infiltrating lymphocytes (TILs) expressing both CTLA-4 and PD-1 were pretreated with anti-PD-1 and anti-CTLA-4 antibodies in vitro, and then were injected intratumorally to the tumor-bearing mice. They found that the adoptive therapy causes tumor regression in 75% mice.

The clinical trials

Nivolumab

Above two preclinical studies both confirmed the effectiveness of combination CTLA-4 inhibitors with PD-1 inhibitors in the tumor rejection, thus researchers decided to further test this treatment strategy, ipilimumab plus nivolumab in the clinical trials (the ongoing and future studies of this regimen are shown in **Table 2**). CheckMate 032 is a phase I/II trial, designed to evaluate the safety and efficacy of nivolumab (nivo) vs. nivo plus ipilimumab (ipi) in solid tumors, including refractory SCLC, and it consists of the initial treatment cohort and the randomized expansion cohort. In the initial part, SCLC patients (pts) who progressed after one or more previous regimens were assigned to receive nivo monotherapy (3 mg/kg Q2W, n=98) or nivo/ipi combination treatment (4 cycles of nivo 1 mg/kg and ipi 3 mg/kg Q3W, then nivo 3 Q2W; n =61). And in the expansion cohort, 247 pts were randomized 3:2 to nivo monotherapy or nivo/ipi combination group. In the 2017 American Society of Clinical Oncology (ASCO) annual meeting [54], Hellmann represented this research team to update the results of the initial treatment arms. An objective response (complete response [CR] or partial response [PR]) by the response evaluation criteria in solid tumors (RECIST) v1.1, as the primary end point, was achieved in 11 (11%) of 98 pts receiving nivo alone and 15 (25%) of 61 pts

in nivo/ipi combination group. According to the latest survival results, nivo/ipi combination showed more potent antitumor activity than nivo monotherapy (median OS 7.9 vs. 4.1 months) and manageable safety profiles (any grade TRAEs occurred in 82% pts, 33% pts suffered grade 3-4 TRAEs and 11% pts discontinued because of intolerant adverse events).

Based on the promising data obtained from Checkmate 032, the phase III trial, CheckMate 451 was designed to evaluate nivo monotherapy, nivo plus ipi followed by nivo monotherapy, and placebo as maintenance therapy for ES-SCLC patients with an ongoing response of stable disease or better after a maximum of 4 cycles of first-line platinum-based doublet chemotherapy [55]. The primary endpoints are OS and PFS. Approximately 810 patients will be enrolled and randomized 1:1:1 to these three cohorts. At present, this trial is active, but not recruiting, and the data collection is expected to be completed in September 2018. The open-label, randomized phase III trial, Checkmate 331 [56], aims to evaluate nivo monotherapy versus single-agent chemotherapy as second-line therapy (topotecan or amrubicin) in relapsed SCLC patients. Estimated 480 patients will be enrolled and randomized 1:1 to nivo or chemotherapy (topotecan in the United States or Europe, and topotecan or amrubicinin Japan, as amrubicin is also approved for SCLC second-line treatment in Japan [57]). The primary endpoint is OS; and secondary endpoints include PFS and ORR. This trial also stops recruiting participants, and its estimated study completion data is November 2019. The other ongoing trials of nivolumab are shown in **Table 2**.

Pembrolizumab

Pembrolizumab is a selective anti-PD-1 antibody approved for the unresectable or metastatic melanoma treatment [58]. It blocks the interaction between PD-1 on T cells and PD-L1 and PD-L2 on tumor cells, thus the level of PD-L1 expression promises to be a potential biomarker which may be positive correlated with patient responses [59, 60]. In the NSCLC treatment, the phase II/III trial, KEYNOTE 010 has showed that there is a positive interrelationship between the PD-L1 levels and the benefits that NSCLC patients could gain from pembrolizumab, and furthermore, patients with PD-L1 expression >50% get significantly larger benefits than the others [61]. Therefore, pembrolizumab has been approved by FDA for treating relapsed NSCLC patients with tumors expressing PD-L1. KEYNOTE-028 is an ongoing phase Ib multi-cohort study evaluating pembrolizumab in treating PD-L1-positive (membranous PD-L1 expression in ≥1% of tumor and associated inflammatory cells or positive staining in stroma), previously treated ES-SCLC patients, and both platinum sensitive and resistance patients were enrolled [62]. 46 of 145 (31.7%) SCLC patients were found to have PD-L1-positive tumors, which suggested a lower prevalence of PD-L1 expression in SCLC compared with NSCLC (23.2% of patients with a score of at least 50% and 37.6% with a score of 1-49%) [60]. 24 participants received pembrolizumab10 mg/kg intravenously every 2 weeks for 2 years, until progression or unacceptable toxicity. Response was assessed by RECIST v1.1 every 8 weeks for the first 6 months and every 12 weeks thereafter, and ORR was 33.3% (95% CI, 16% to 55%) with one CR and 7 PR. The median PFS and OS were 1.9 months (95% CI, 1.7 to 5.9 months) and 9.7 months (95% CI, 4.1 months to not reached), respectively. The authors looked at the potential relationship between higher level of PD-L1 expression and the frequency of responses and there was no significant difference found (p=0.235) [63].

There are several ongoing clinical trials that evaluating the efficacy and safety of pembrolizumab in SCLC treatment regardless of PD-L1 expression (**Table 2**). In the 2017 ASCO annual meeting, Shirish reported a phase II study of pembrolizumab as maintenance therapy in ES-SCLC patients who had CR, PR or stable disease (SD) after 4-6 cycles of PT-DC [64]. Of the 45 patients, the disease control rate was 42% (1 CR, 3 PR and 15 SD), and at a median follow up of 6 months, the median PFS was 1.4 months (90% CI, 1.3-4.0 months), PFS according to immune related response criteria (irPFS) was 4.7 months (90% CI, 1.8-6.7 months), and the median OS was 9.2 months (90% CI, 6.1-15.2 months). According to the randomized, doubled-blind, placebo-controlled clinical trial CALGB 30504, the median PFS and OS of ES-SCLC patients whose initial chemotherapy followed by placebo maintenance were 2.1 months (70% CI, 1.27 to 2.08 months) and 6.9 months (95% CI, 0.79 to 2.10 months), respec-

Table 3. The efficacy and safety of several common regimens as second-line treatment in SCLC patients

Regimen (iv.)	Population (n)	ORR (%)	Median PFS (95% CI)	Median OS (95% CI)	TRAEs (>Grade 3)	Ref.
Nivo 3 mg/kg Q2W	SCLC (98)	11.2	1.4 months (1.4-1.6 months)	4.1 months	14.3%	[54]
Nivo 1 mg/kg+ipi 3 mg/kg Q3W→nivo 3 mg/kg Q2W	SCLC (61)	24.6	2.8 months (1.4-4.4 months)	7.9 months	32.8%	[54]
Pembro 10 mg/kg Q2W	SCLC with PD-L1 expression (42)	33.3	1.9 months (1.7-5.9 months)	9.7 months (4.1 to not reached)	Bilirubin elevation (1/24) colitis/intestinal ischaemia (1/24)	[62]
Topotecan 1.5 mg/m^2/d D1-5 Q3W	SCLC sensitive to initial CT (151)	21.9	14.6 weeks (13.3-18.9 weeks)	35.0 weeks (31.0-37.4 weeks)	Leukopenia (75.3%) Neutropenia (87.8%) Thrombocytopenia (43.3%) Anemia (30.7%)	[10]
Amrubicin 40 mg/m^2/d D1-3 Q3W	Refractory SCLC (75)	21.3	3.2 months (2.4-4.0 months)	6.0 months (4.8-7.1 months)	Neutropenia (66.7%) Thrombocytopenia (40.6%) Leukopenia (34.8%) Fatigue (21.7%)	[67]

SCLC: small-cell lung cancer; *ES-SCLC:* extensive-stage SCLC; *LS-SCLC:* limited-stage SCLC; *nivo:* nivolumab; *ipi:* ipilimumab; *CT:* chemotherapy; *OS:* overall survival; *PFS;* progression-free survival; *ORR;* objective response rate; *pembro:* pembrolizumab; *DoR:* duration of response; *CI:* confidence interals; *TRAEs:* treatment-related adverse events.

tively [65]. Thus the researches drew a conclusion that maintenance pembrolizumab can not improve PFS, but favorable OS showed that some ES-SCLC patients could benefit from this regimen, and they analyzed the tumor tissue of 35 (78%) patients for PD-L1 expression, which was positive (any level of expression was considered as positive) in one patient, and no clear association observed between them. Moreover, in CheckMate 032, PD-L1 expression was assessable in 148 (69%) of 216 patient samples, 25 (17%) had >1% PD-L1 expression, and 7 (5%) had >5% PD-L1 expression, and the pre-planned exploratory analysis showed that tumor responses occurred in patients irrespective of PD-L1 expression [66].

Conclusion

There is still no standard second-line therapy for the SCLC patients whose diseases relapse after the first-line platinum-based doublet chemotherapy, as topotecan and amrubicin with disappointing disease control rate and serious TRAEs. With the rapid development of immunotherapy, especially the immune checkpoint blockade, the scientists wonder if this novel cancer treatment strategy can change the treatment paradigm for SCLC and provide more options for these patients. Preclinical researches about the combined inhibition of PD-1 and CTLA-4 have shown its promising antitumor activity, especially when tumor recombinant vaccinations added. According to the preliminary outcomes from the clinical trials, durable responses and manageable TRAEs were observed in PD-1 inhibitors, nivolumab and pembrolizumab treatment, compared with the conventional second-line chemotherapy (**Table 3**, [10, 54, 62, 67]). However, it is lack of sufficient evidence supporting that PD-1 blockade could prolong survival for SCLC patients and moreover, the role of PD-L1 as a potential biomarker still remains unclear, so we are looking forward to the survival data of the ongoing phase III clinical trials (**Table 2**), and further researches are really necessary to ascertain the long-term safety and efficacy of PD-1 inhibitors.

Disclosure of conflict of interest

None.

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