REVIEW ARTICLE

Challenges for the development of immunotherapy in small-cell lung cancer

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Abstract

Small-cell lung cancer (SCLC) is a clinically aggressive cancer, and accounts for 15% of all types of lung cancer. SCLC is characterized by its rapid growth, early dissemination, and easy acquisition of multidrug resistance to chemotherapy. While most of the patients with SCLC are eligible to systemic chemotherapy owing to the presence of distant metastasis at the time of diagnosis, the median survival with the standard chemotherapy is less than 12 months. Numerous clinical trials, including molecular targeted therapy, have been conducted in hopes of developing a novel therapeutic strategy in SCLC, but eventuated disappointed results. Consequently, the standard chemotherapeutic regimen has not changed for three decades. Moreover, clinically beneficial therapeutic strategies for patients with relapsed-SCLC are extremely limited. Thus, effective treatment of SCLC has been leveling off in spite of the recent dramatic progress in the treatment of non-SCLC. Genomic analysis revealed that definitively targetable molecules with oncogenic driver activity were rarely detected

in SCLC. Therefore, immunotherapy rather than molecular targeted therapy is considered to be promising in the improvement of prognosis in patients with SCLC. Immune checkpoint inhibitors (ICIs) such as nivolumab and pembrolizumab have been approved for the treatment of the patients with non-SCLC, and have dramatically improved their prognosis. These ICIs exert antitumor effect via activating adaptive immunity. Some clinical trials have demonstrated promising effects of ICIs in the treatment of relapsed-SCLC. We have reported that trastuzumab, a humanized anti-human epidermal growth factor receptor 2 antibody, could exert remarkable antitumor effects against SCLC mainly through antibody-dependent cell-mediated cytotoxicity (ADCC) in preclinical models and clinical settings. ADCC is commonly recognized as one of the best ways to activate innate immunity. It is essential to clarify how to maximize the benefit of the immunotherapy in order to improve the prognosis of SCLC.

Keywords: immunotherapy, small-cell lung cancer, immune checkpoint inhibitor, antibody dependent cell-mediated cytotoxicity

1. Introduction

Small-cell lung cancer (SCLC) accounts for 15% of all the histological subtypes of lung cancer. SCLC is characterized by rapid growth and early dissemination, and thus more than 70% of the patients with SCLC already have distant metastasis at the time of diagnosis. These patients are regarded to have extensive-stage disease (ED-SCLC), and are eligible to systemic chemotherapy. Combination therapy of platinum plus etoposide or irinotecan is commonly used as the standard chemotherapeutic regimen of SCLC.[1, 2] Despite of the high initial response rate of about 75% in SCLC, the outcome is extremely poor with a median overall survival (OS) of less than 12 months because of the high recurrence rate along with the acquisition of multidrug resistant phenotype.[1] Although various kinds of clinical trials, including those that evaluate antitumor efficacy of molecular targeted therapy, has been conducted, the results have been disappointing.[3] One of the reasons of these unsatisfactory results of molecular targeted therapy is that definitively targetable molecules with oncogenic driver activity are rarely detected in SCLC.[4] Consequently, the standard chemotherapeutic regimen remains unchanged since it was established three decades ago. The development of novel therapeutic strategy in SCLC is a pressing issue.[5]

Antitumor effect of cancer immunotherapy has been clinically validated in several types of cancers.[6] In lung cancer, immune checkpoint inhibitors (ICIs) brought about the dramatic improvement in the treatment of non-SCLC.[7, 8] Immunotherapy

may have a potential to be a breakthrough therapy in SCLC also. In this article, we review the possibility of various types of immunotherapy for the treatment of SCLC.

2. Mechanisms of escape of SCLC cells from immune system

There are many mechanisms for SCLC cells to escape from the immune system. Immune system recognizes cancer cells with their tumor-related antigens. First, these tumor-related antigens are captured by antigen-presenting cells (APCs) such as dendritic cells (DC). Next, they are bound to major histocompatibility complex (MHC) class II molecule, and are presented to T cells. Thereafter, T cells can recognize cancer-related antigens as 'non-self' and eliminate cancer cells.[9] In SCLC, maturation and function of DC is inhibited by a number of soluble and membrane-bound molecules.[10] Moreover, previous studies have demonstrated that expressions of MHC class I and II indispensable for T cell recognition, were also decreased.[11, 12] In fact, we observed the loss of MHC class I expression in human SCLC tumor specimens.[13] By means of these mechanisms, SCLC may escape from T cell immune surveillance.

3. Immune checkpoint inhibitors

Multiple proteins such as cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1) and its ligand and programmed cell death-1 ligand-1 (PD-L1), PD-L2, B7, lymphocyte-activation gene-3 (LAG-3) and T cell immunoglobulin and mucin domain-containing molecule-3 (TIM-3) have been identified as immune checkpoint proteins.[14] Among these proteins, CTLA-4, PD-1 and PD-L1 were clinically proved to be promising candidates for the treatment of melanoma and other malignancies.[15] Two anti-PD-1 antibodies, nivolumab and pembrolizumab, have been approved for the treatment of non-SCLC as ICIs.[16] They block inhibitory receptor on tumor-infiltrating T cells and regulate the adaptive immune system to exert antitumor response.[17] The first trial to have verified the efficacy of ICIs in relapsed-SCLC is CheckMate032 (Table 1). This was a multicenter, multi-arm, open-label phase I/II study to evaluate the antitumor activity of nivolumab and nivolumab plus ipilimumab, anti-CTLA-4 antibody, in relapsed-SCLC. The primary endpoint of this study was objective response rate. Patients were randomized and treated with nivolumab 3 mg/kg monotherapy every two weeks (n = 98), nivolumab 1 mg/kg plus ipilimumab 3mg/kg every three weeks (n = 61), or nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every three weeks (n = 54). In the respective three arms, the objective response rate was 10%, 23%, and 19%, and the median (OS) was 4.4 months, 7.7 months, and 6.0 months, respectively.[18] Therapeutic option for the patients with relapsed-SCLC is extremely limited under

the practical circumstances. Topotecan monotherapy is the only approved regimen in U.S. and Europe for the treatment of relapsed-SCLC. The response rate and median OS of topotecan monotherapy in relapsed-SCLC was reported to be 16.9% and 7.8 months, respectively.[19] Compared to the historical data, the authors considered the response rate and OS obtained by ICI treatment in CheckMate032 to be durable. To confirm the efficacy of nivolumab, a phase III trial of nivolumab vs. chemotherapy (topotecan in the US or EU, and topotecan or amrubicin in Japan) in patients with relapsed-SCLC is currently being conducted (CheckMate331).

Table 1. Clinical trials testing ICIs for the treatment of relapsed-SCLC

Study (Trial ID)	ICIs	Phase	No.	ORR (%)	Median PFS (Months)	Median OS (Months)	Ref
CheckMate032 (NCT01928394)	Nivolumab 3mg/kg, q2w	I/II	98	10	1.4	4.4	16
	Nivolumab 1mg/kg plus ipilimumab 3mg/kg, q3w		61	23	2.6	7.7	
	Nivolumab 3mg/kg plus ipilimumab 1mg/kg, q3w		54	19	1.4	6.0	
KEYNOTE-028 (NCT02054806)	Pembrolizumab 10mg/kg, q2w	Ib	24*	33	1.9	9.7	18

Abbreviations: ICI, immune checkpoint inhibitor; ORR, objective response rate; PFS, progression free survival; OS, overall survival; Ref, reference

* Tumor PD-L1 expression defined by membranous PD-L1 expression > 1% of tumor or positive staining in stroma was required to be eligible for treatment.

The antitumor effects of another anti-PD-1 antibody, pembrolizumab, against relapsed-SCLC were also evaluated in multicohort phase Ib open-label study KEYNOTE-028 (Table 1). In this study, only patients with PD-L1-positive (tumor proportion score > 1%) relapsed-SCLC were enrolled. Eligible patients (n = 24) were treated with pembrolizumab 10 mg/kg every two weeks. Response was observed in eight patients (33%) including one complete response, and median OS was 9.7 months. From these results, patients with PD-L1-expressing relapsed-SCLC seem to response well to pembrolizumab.[20] However, as the au-

thors of this study mentioned, it is too early to regard PD-L1 as a biomarker to select the candidates of pembrolizumab in the treatment of relapsed-SCLC because objective response to nivolumab was observed independently of PD-L1 expression in CheckMate032.[18] Furthermore, besides the lower frequency of PD-L1 positivity in SCLC compared with that of non-SCLC, immunohistochemistry analysis revealed that expression of PD-L1 in SCLC specimens was rarely detected on tumor cells themselves but was mainly detected on macrophages in the stroma.[21-23] As an alternative biomarker to PD-L1, tumor mutation burden (TMB) was recently reported to be a promising biomarker to decide the eligible patients of ICI treatment in SCLC. In CheckMate032 trial, the response rate of the patients with high TMB is twice as high as compared to that of the patients with average level of TMB.[24] As the number of TMB increase, the number of "non-self" antigen increase, which enhances the sensitivity to ICIs.[25, 26] Additionally, SCLC has a very high mutational burden because tobacco exposure is involved in its carcinogenesis.[27, 28] From these points of views, TMB is considered to be a reasonable biomarker in SCLC.

Despite of the promising results of ICIs for the treatment of relapsed-SCLC, addition of ipilimumab to chemotherapy did not improve the OS compared to chemotherapy alone in chemotherapy-naïve patients with ED-SCLC (median OS was 11.0 months for chemotherapy plus ipilimumab vs. 10.9 months for chemotherapy plus placebo) (Table 2).[29] Although the reasons of this discrepancy in the sensitivity to ipilimumab between chemo-naïve and relapsed-SCLC remain unknown, we presume that differential level of MHC class I expression between chemo-naïve and relapsed-SCLC is one possible mechanism. While MHC class I is absolutely necessary for cytotoxic T cells to recognize the tumor antigen on target tumor cells, expression of MHC class I has been reported to be remarkably attenuated in SCLC, which is beneficial for SCLC cells to escape from T cell surveillance.[12, 13, 30] On the other hand, it was reported that chemotherapeutic drugs induced MHC class I expression.[31] Therefore, once SCLC cells are exposed to cytotoxic drugs, they may become more sensitive to cytotoxic T cells via upregulation of MHC class I expression. Several phase III clinical trials to investigate the efficacy of other ICIs in first-line treatment of ED-SCLC are currently ongoing. For example, nivolumab is being evaluated in CheckMate451 as maintenance therapy after first-line treatment by comparing the efficacy of nivolumab alone, nivolumab plus ipilimumab, or placebo. Meanwhile, additional effect of pembrolizumab in combination with chemotherapy is now under investigation in KEYNOTE-064.[32] Conclusive data on whether ICIs are active for the first-line treatment of ED-SCLC is not available until the final results from these phase III trials are reported.

Table 2. Clinical trials testing ICIs as first line treatment of SCLC

Trial ID	Study detail	Phase	No.	ORR (%)	Median OS (Months)	TRD	Ref
NCT00527735	Platinum and etoposide plus placebo	III	476	62	10.9	2%	27
	Platinum and etoposide plus ipilimumab 10mg/kg, q3w		478	62	11.0 HR 0.94; 95% CI, 0.81 – 1.09 *P* = 0.3775	18%	

Abbreviations: TRD, treatment-related discontinuation; HR, hazard ratio; CI, confidence interval

4. Vaccines

Cancer vaccines are designed to present tumor antigen to adaptive immune system, thereby enhancing antitumor killing effect of T cells. Cancer vaccines are recognized to be suitable for the treatment of minimal residual disease.[33] Most of the patients with SCLC respond well to initial chemotherapy, where the tumor dramatically shrinks. Cancer vaccines may exert antitumor effects against the residual tumor after initial chemotherapy. Therefore, patients with SCLC after induction therapy are thought to be reasonable candidates for cancer vaccines. Bec2 is an anti-idiotypic antibody that mimics glycosphingolipid antigen GD3 which was shown to be overexpressed in 60% of SCLC tissue specimens.[34] Bec2 can induce anti-tumor immunity against selectively targeting GD3. Additionally, Bacille Calmette-Guerin (BCG) is also able to augment the immune reaction of Bec2 when administered simultaneously.[35] A small pilot study showed survival benefit in patients with SCLC treated with Bec2/BCG vaccine after induction therapy.[36] Based on the favorable result of the pilot study, a large phase III study to evaluate the efficacy Bec2/BCG vaccination was conducted. Although humoral response was observed in 71 of 216 (33%) patients assessed, statistically significant improvement of survival was not observed in patients who were vaccinated in this phase III study.[37] Another clinical study was performed to evaluate the efficacy of DC-based p53 vaccine. In SCLC, *p53* gene is almost universally mutated. Mutant p53 protein has a prolonged half-life, which results in abundant accumulation in tumor cells.[28, 38] DC-based p53 vaccine was designed to enhance the tumor antigen presentation of DC by transduction

of *p53* gene using adenoviral vector. Although antitumor effect of this vaccine was not observed in patients with progressive disease after initial chemotherapy, a high rate of objective clinical responses to subsequent chemotherapy was observed. This result suggests that this vaccine may work as a sensitizer to chemotherapy.[39] The fundamental problem in cancer vaccine therapy is the difficulty in selecting an appropriate targeting molecule. A recent phase II study suggested that personalized peptide vaccination (PPV) had a potential to prolong OS in patients with previously treated SCLC.[40] Also in this study, 32 of 46 patients (70%) received chemotherapy during PPV. These studies indicate that treatment with cancer vaccines is insufficient for disease control as monotherapy, and should be only considered in combination with systemic chemotherapy in SCLC.

5. Cellular immunotherapy (CIT)

Natural killer (NK) cells detect the absence of MHC class I, recognize the cells as "non-self", and exert cytolytic activity. Therefore, SCLC cells with low expression of MHC class I are considered to be sensitive to NK cell-mediated cytolysis. Lymphokine-activated killer (LAK) cells generated from autologous lymphocytes by exposing to interleukin (IL)-2 are heterogeneous population of cells consisting of NK, NKT and T cells. They are also expected to be effective for the treatment of SCLC because they can exert cell lytic effects independently of MHC class I restriction.[41] Indeed, several in vitro studies had revealed that SCLC cells were susceptible to NK/LAK-mediated cell lysis.[42, 43] In fact, infusion of cytokine-induced killer (CIK) cells clinically showed promising anticancer effect.[44, 45] CIK cells are generated by interferon-δ, anti-CD3 antibody, IL-1 and IL-2.[46] These cells represent a heterogeneous cell population consisting of $CD3^+CD56^+$ cells, minor fraction of typical T cells ($CD3^+CD56^-$), and NK ($CD3^-CD56^+$) cells, and also have non-MHC-restricted cytolytic activity against cancer cells.[47] A pilot study to evaluate the efficacy of CIT with autologous NK, γδT, and CIK cells proved that CIT significantly improved OS in patients with SCLC when administered as maintenance therapy after the standard first-line treatment (median OS 20 vs. 11.5 months, P = 0.005).[44] Moreover, another study reported that combination therapy of chemotherapy and CIK transfusion prolonged progression free survival compared to chemotherapy alone in late stage patients with SCLC (median PDS 8 vs. 4 months, P = 0.005).[45] Thus, CIT may become a novel option for the treatment of SCLC, but further multi-center randomized studies are needed to confirm the efficacy.

6. Antibody-dependent cell-mediated cytotoxicity (ADCC)

ADCC is the mechanism by which effector innate immune cells such as NK cells, macrophages, DC, and granulocytes lyse the antibody-coated target cells through the

release of lytic granules.[48] Previous findings of the high sensitivity of SCLC cells to NK cell-mediated cell lysis encouraged us to examine the efficacy of ADCC for the treatment of SCLC. Moreover, several tumor-specific monoclonal antibodies have recently shown clinical benefits through ADCC mechanism in various types of cancers.[49] Among them, we examined the antitumor efficacy of trastuzumab, a humanized anti human epidermal growth factor 2 (HER2) antibody, in preclinical model and clinical settings. The reason why we focused on HER2 is that HER2 expression was not only reported to be an independent negative prognostic factor especially in ED-SCLC but also was found to be upregulated when HER2-positive SCLC cells acquired chemoresistance.[50, 51] As expected, trastuzumab induced ADCC against HER2-positive chemoresistant SCLC cells *in vitro* and *in vivo*. Notably, trastuzumab-mediated ADCC was augmented by the co-expression of intercellular adhesion molecule (ICAM)-1 which is one of the required molecules for NK cells to lyse the target cells (Figure).[52-54] From these preclinical results, we performed irinotecan plus trastuzumab combination therapy in two patients with HER2-positive relapsed-SCLC, and observed favorable response in both patients (one patient achieved partial response and the other achieved stable disease).[13] We also have recently reported that trastuzumab emtansine (T-DM1), an antibody drug conjugated anti-HER2 antibody, could inhibit growth of irinotecan-resistant HER2-positive SCLC mice xenografts which are refractory to trastuzumab-mediated ADCC due to lack of ICAM-1 expression.[55] Our observations suggest that ADCC is expected to be a promising mechanism for the treatment of SCLC. However, not only the expression of antibody-targeted molecule but also the expression of co-activators including ICAM-1 may be needed to exert maximum ADCC activity. Further investigation is necessary to identify the reliable co-activators to select the candidates for ADCC-based treatment. Therefore, at this point, not all of the patients with SCLC are eligible for antibody therapy based on ADCC mechanism. Antibody-drug conjugates (ADCs) are reasonable therapeutic options. Most of the licensed therapeutic antibodies have a human IgG1 fragment crystallisable (Fc) domain which has a high affinity to activating Fcγ receptors (FcγRI and FcγRIII) on effector cells.[56] Rovalpituzumab tesirine is also an ADC consisted of delta-like protein 3 (DLL3)-specific antibody and DNA cross-linking agent SC-DR002 showed encouraging antitumor effects in previously treated patients with SCLC. The response rate was 18% (11 of 60 assessable patients), and median progression free survival (PFS) was 3.1 months. Moreover, median PFS tends to be longer in patients with high expression of DLL3 (4.5 months).[57] To some extent, ADCC is expected to be involved in the antitumor effects of rovalpituzumab because it also has an IgG1 Fc domain which enables it to induce ADCC activity.

Figure

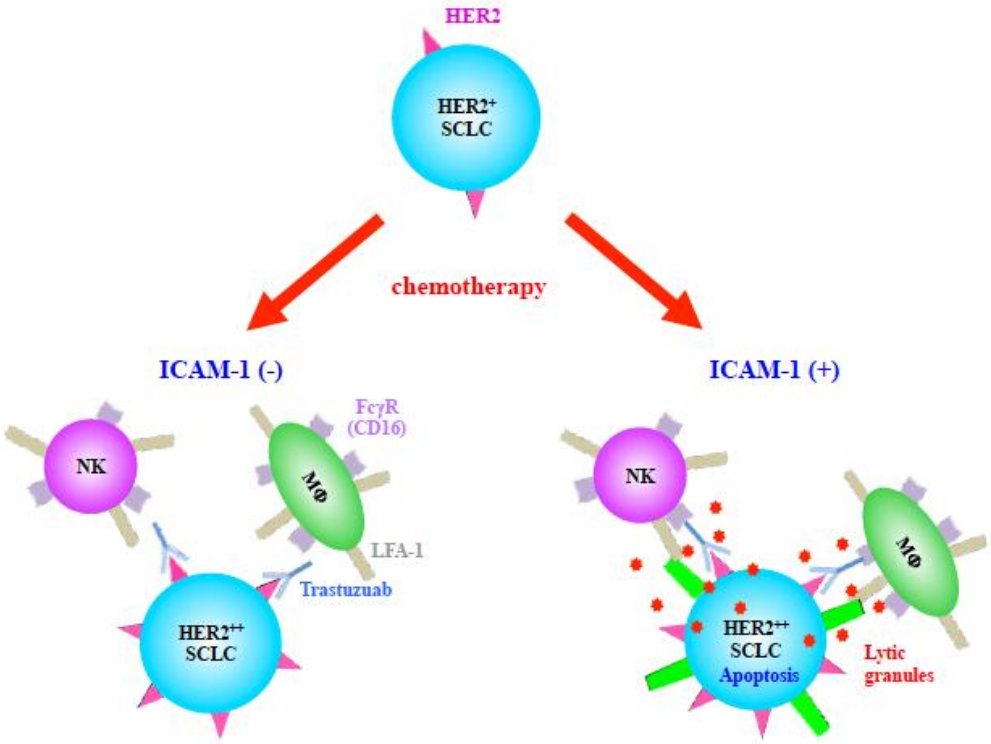

Figure. Trastuzumab induces ADCC against HER2-upregulated chemoresistant SCLC cells.

HER2 expression is upregulated once HER2-positive SCLC cells acquire chemoresistance. Trastuzumab exerts anti-tumor effects against HER2-overexpressing cells via inducing ADCC activity. Therefore, HER2-upregulated chemoresistant SCLC cells are considered to be good targets of trastuzumab-based therapy. However, co-activator is indispensable to maximize traszumab-mediated ADCC. ICAM-1 works as a strong facilitator of trastuzumab-mediated ADCC through promoting adhesion between effector and target cancer cells by engagement with LFA-1. (modified from Minami T et al., 2013)

Abbreviations: NK, natural killer; MΦ, macrophage; ICAM-1, intercellular adhesion molecule-1, LFA-1, lymphocyte function associated antigen-1

7. Conclusion

Numerous challenges lay ahead in the establishment of immunotherapy in SCLC. According to the previous studies and our experimental results, SCLC is extremely difficult to control by any single kind of immunotherapy or immunotherapy alone. First, SCLC can evade the adaptive immune system by greatly reducing MHC class I expression, which is also expected to cause resistance to ICIs. In addition, the most effective usage of non-MHC-restricted innate immune system remains uncertain. While ADCC is considered to be a promising mechanism, in order to kill SCLC cells effectively not only is the antibody targeting protein of importance, but also essential co-activator proteins such as ICAM-1 are needed. Thus, it is very important to take

co-activator proteins into consideration for maximizing ADCC activity. However, identifying the reliable co-activator proteins has proven to be difficult because various stimulations, including chemotherapy and cytokines derived from tumor microenvironment, often affect the expression of these co-activator proteins. Further studies are indispensable to optimize the timing for utilizing ADCC activity. Second, complex heterogeneity in SCLC causes trouble in identifying the appropriate tumor antigen and targeted molecule in cancer vaccine and antibody-based therapy, respectively. Third, the growth speed of the tumors in patients with SCLC is so rapid that there is not enough time to wait until immunotherapy fully exerts its antitumor potential. From these aspects, we should consider that it is necessary not only to orchestrate both innate and adaptive immune system, but also to perform combination therapy with cytotoxic agents or to utilize ADCs when using immunotherapy in SCLC.

Acknowledgements:

We would like to thank the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (18K15962; T.M.) for granting permission to publish this article.

Competing financial interests:

T.K. received lecture fee from Chugai Pharmacology Co. Ltd. The other authors declare no competing financial interests.

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