LAG-3 and PD-1/PD-L1 Inhibitors Might Become a Promising Treatment for Small Cell Lung Cancer

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Abstract

Background Immune therapy has achieved notable success in cancer treatment. A novel immunocheckpoint, Lymphocyte activating gene-3 (LAG-3), has shown promising therapeutic efficacy in non-small cell lung cancer (NSCLC). However, literature about LAG-3 in small cell lung cancer (SCLC) is scarce. We statistically analyzed the correlation of LAG-3 expression with programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1), its survival predictive significance, and the possibility of becoming a treatment alternative of SCLC.

Methods In this study, we included 102 patients diagnosed with SCLC. The expression of protein was evaluated by immunohistochemistry (IHC) staining. We performed correlation analysis and survival analysis with SPSS software (version 17.0; SPSS, Inc.; Chicago, IL)

Results In SCLC, none tumor cell expressed LAG-3. The expression of LAG-3 was remarkably associated to PD-1 and PD-L1 expression on TILs ($p=0.006$, $p=0.001$). LAG-3 was the only prediction for TILs PD-L1 expression (ORs=0.161, 95% CI: 0.063-0.412, $p<0.01$). Although LAG-3-positive patients had relatively longer RFS, LAG-3 expression had no statistically significant differences in predicting prognosis ($p=0.088$).

Conclusions We found a significant correlation of LAG-3, PD-1, PD-L1 expression. LAG-3 is an important immune checkpoint closely linked to PD-1/PD-L1 and might be a promising novel alternative of immune therapy for SCLC.

Background

Among all cancer types, lung cancer ranks first in both morbidity and mortality and poses an increasingly serious threat to human health^[1,2]. Approximately 10% to 15% cases can be categorized as small-cell lung cancer (SCLC), a cancer type with high growth fraction, high recurrence rate, leading to poor prognosis^[3-5]. Although chemotherapy is the standard first-line treatment for SCLC^[6], resistance to chemotherapy hinders survival prolongation. Therefore, it is an urgency to explore some more effective therapeutic strategies for patients with SCLC.

Some tumor cells with less immunogenicity can escape from immune elimination and developed into cancers, which was reported can be reversed by suppressing certain immune checkpoints^[10]. The notable success of some immune checkpoints inhibitors in treating cancers has been reported^[11,12]. The PD-1/PD-L1 pathway inhibitor has been approved effective for treating NSCLC. It could also lead to significantly longer survival in SCLC patients with the first-line chemotherapy combined^[13-16].

Whereas the insensitivity to PD-1/PD-L1 blockade hinders its extended use^[17,18], some other alternatives are now at the forefront of research, such as LAG-3^[19], a novel immune inhibitory checkpoints^[20].

LAG-3, a surface molecule, also known as CD 223, was identified first in 1990^[21]. LAG-3 is expressed on the membrane of various immunocytes, including tumor-infiltrating lymphocytes (TILs), dendritic cells, T regulatory cells (Treg), natural killer cells, B cells, etc.^[22,23] As a member of immunoglobulin superfamily, LAG-3 is structurally similar to CD4, with approximately 20% homology share at DNA sequence^[24]. Likewise, LAG-3 shows stronger affinity to human leukocyte antigen II (HLA-II) expressed on antigen presenting cells (APCs) compared to CD4, thereby prohibits the binding of HLA-II with TILs, hindering anti-tumor response^[25,26]. In HLA-II-positive melanoma tumors, this might pave the way for immune escape with bidirectional function^[23].

LAG-3 serves as an essential marker of T cell exhaustion, promoting T-cell apoptosis, inhibiting proliferation, suppressing activation, declining cytokines secretion and increasing tolerance^[27,28]. Elevated LAG-3-expression was observed on TILs of patients with various solid tumors, such as hepatocellular carcinoma, gastric carcinoma etc, as well as hematologic malignancies^[29].

Scholars regarded that LAG-3 has co-function with PD-L1 and PD-1^[11]. In vivo research suggested that T cells would get activated if one of the pathways was blocked. Strategy of blocking both pathways showed even more notable effect^[30]. LAG-3 may become a new promise immune checkpoint in cancer treatment. Additionally, both LAG-3 and PD-1 pathways could make a promising therapeutic strategy better than CTLA-4 blockade for less toxicity^[11]. Moreover, soluble LAG-3 can possibly be promising anti-cancer vaccine^[31].

Similar to other types of cancer, our recent study has found that some NSCLC patients had LAG-3-positive TILs. The expression of LAG-3 can be predicted by PD-1 expression and related to worse prognosis^[23]. However, literature is scarce on the LAG-3 expression and how it effects survival in SCLC. In this study, we enrolled 102 patients with SCLC, investigated LAG-3 expression in TILs, conducted survival analysis and correlation analysis of clinic pathological traits and PD-L1, PD-1, LAG-3 expression.

Patients And Methods

1. Patients

We included 102 patients aged from 38 to 81 who were diagnosed as SCLC in Shanghai Pulmonary Hospital from March, 2017 to January, 2019. We reviewed the surgical histology reports and categorized lung cancer stages with 8th edition International Association for the Study of Lung Cancer (IASLC) TNM staging system. Fifty four of them received chemotherapy after diagnosis. All participators were competent to provide consent.

2. IHC Procedure

Dewaxing tissue slides with xylene, then alcohol, rinsing slides with distilled water. After recovering antigen, background staining reduction, we incubated the primary antibody in the slides at RT for 1 hour.

And then, rinsing with PBS, incubating with the HRP-conjugated goat anti-rabbit immunoglobulin G detective antibody at RT for 30 min. Rinsing with PBS, visualizing the antigen with DAB, cell nuclear counterstain with hematoxylin and slides mounting. IHC was performed by pathologist Liping Zhang.

3. Cutoff value determination of surface markers

We chose a value of at least 5% as LAG-3 cutoff for the best prediction of recurrence-free survival (RFS). The PD-1 on TILs staining was determined as positive when over 1%. Five percent staining was determined as lower limit of PD-L1 cutoff. The staining of FOXP3, CD3, CD4, CD8 was confirmed as positive when more than 10%, 40%, 30%, 30% individually.

4. Statistical analysis

We conducted correlation analysis and survival analysis with SPSS software (version 17.0; SPSS, Inc.; Chicago, IL). Spearman's rank correlation was applied to calculate relativity of PD-L1, PD-1, LAG-3 expression. We used Chi-square tests to evaluate the relativity of clinicopathological traits and PD-L1, PD-1, LAG-3 expression. Logistic regression model was applied to figure out whether LAG-3 made meaningful expression prediction for PD-L1 and PD-1, gender, age, smoking status, lung cancer stage, history of chemotherapy were all included as well. Kaplan-Meier method was implemented to estimate survival curves, and Cox regression model to the correlation analysis on RFS and clinical features, including age, gender, history of chemotherapy, smoking status, staging of lung cancer, PD-L1 on tumor cells, PD-L1 on TILs, PD-1, LAG-3 on TILs and CD3, CD4, CD8, FOXP3. Statistical significance was defined as $P < 0.05$. All statistics were 2-sided.

Results

1. Characterization of LAG-3 expression

Forty patients had LAG-3-positive TILs (39.2%). None tumor cells were observed LAG-3-positive.

2. Patient Characteristics

The total number of patients enrolled was 102, among them, 84 (82.4%) were female and 18 (17.6%) were male. The median age was 62 years old. Fifty eight (56.9%) of them were non-smoker. Fifty four (52.9%) patients received chemotherapy before. All the patients were diagnosed as SCLC, with 38 patients (37.3%) at stage I, 22 (21.5%) at stage II, and 42 (41.2%) at stage III (Table 1).

3. Correlation of LAG-3, PD-1, PD-L1 Expression

Statistical significance was found between PD-L1 on TILs and LAG-3 expression and ($p=0.001$), which was also correlated with on TILs PD-1 expression ($p=0.006$). No significant correlation existed between PD-L1 on tumor cells and LAG-3 ($p=0.365$) (Table 2). Based on univariate and multivariate analysis by logistic regression model, we found that LAG-3 was of a certain significance in the prediction of TILs PD-

L1 expression (ORs=0.161, 95% CI: 0.063-0.412, $p<0.01$). LAG-3, lung cancer stages and history of chemotherapy all made meaningful predictions for PD-1 expression on TILs (Table 3, Table 4).

4. Survival analysis

With Kaplan-Meier analysis, patients who were LAG-3-positive had longer RFS, but there was no significant statistical difference in comparison with LAG-3-negative patients ($p=0.088$) (Figure 1).

5. Cox Regression Analysis of RFS

Revealed by the results, lung cancer stage was the only prediction of RFS ($p = 0.021$, ORs=1.885, 95% CI: 1.102-3.223). PD-L1, FOXP3, CD3, CD4 and CD8 had significance in Cox regression univariate analysis ($p=0.006, 0.004, 0.007, 0.010, 0.007$ individually), but had no significance in multivariate Cox regression (Table 5.).

Discussion

As a novel immune checkpoint, literature is scarce on LAG-3 expression in SCLC and the correlation with survival. To our knowledge, this is the first indication of the possible immunotherapeutic effect for SCLC focused on LAG-3.

As is stated above, LAG-3 serves as an essential marker of T cell exhaustion^[27,28]. TILs are regarded as crucial components in anti-tumor immune response and directly related to the development of cancer^[32]. The function of CD4+, CD8+ T cells, DCs, Tregs etc. is regulated by inhibitory and active receptors, remarkably impact cancer immune escape^[33].

From a mechanistic standpoint, LAG-3 blockade is equal to the blockade of the binding between LAG-3 and HLA-II molecules, which increases the binding of HLA-II to TILs, thus enhances anti-tumor responses^[34]. High LAG-3 expression can be observed on TILs in hematologic malignancies and various solid tumors, including hepatocellular carcinoma, gastric cancer, renal cell carcinomas and ovarian cancer^[29]. In our study, LAG-3-positive TILs existed in 39.2% of patients; with none tumor cells expressed LAG-3. Additionally, research indicated that if PD-1 or LAG-3 pathway alone was blocked, TILs would get more activated and lead to prolonged survival, either by antibodies or knocking down^[35]. Built on existing studies, we used to consider LAG-3 a novel alternative of immune-based treatment for cancers. In our recent study, we found that NSCLC patients with LAG-3-negative TILs had longer survival^[23]. But in SCLC LAG-3 could not predict survival.

Immune escaping pathways are closely associated with one another^[36]. LAG-3 is co-expressed on TILs with PD-1 and have remarkable synergy with it, disrupting immune responses to cancer cells^[37]. Based on research before, compared with blocking either LAG-3 or PD-1 alone, blocking both pathways showed much more remarkable therapeutic efficacy for cancers^[30, 35]. In the meanwhile, due to the upregulated expression of LAG-3 in patients insensitive to PD-1 blocking treatment, application of combined strategy

will improve the prognosis^[29,36]. This has already been proved in treating melanoma patients with this condition^[38,39].

Given the different impact the above-mentioned checkpoints on NSCLC and SCLC survival, we take the immune mechanism into consideration. Different from over-expressed on variety of tumors including NSCLC, PD-L1 expression is suppressed in SCLC^[40]. In our study, there were only 4 patients (3.9%) with PD-L1-positive tumor cells, consistent with previously reported results in an immunohistochemical study of SCLC which found PD-L1-positive tumor cells was lower than 20%^[41]. This might be one of the reasons why PD-1/PD-L1 pathway inhibition therapy alone cannot lead to notably improved prognosis in SCLC^[42]. On the other hand, worse outcomes of SCLC was also considered to linked with higher FOXP3+T cells infiltrates^[43]. Furthermore, the immune microenvironment of SCLC seems to be distinct from that of other solid tumors, for instance, NSCLC^[40]. In this context, we can infer that combined immunotherapy is especially important in SCLS.

There were some limitations should be taken into consideration. Firstly, this study was a retrospective one. Secondly, the sample size was not large enough and more data are needed to support our indication.

Researchers are moving forward in the investigation of immunotherapy in recent years, aiming to more favorable prognosis for patients with SCLC. It is noteworthy that the characteristics of the SCLC immune microenvironment remain unclear. Researchers are moving forward in LAG-3 function and its interaction with other immunomarkers, yet there are still questions remain to work out. What role does LAG-3 play in the development of SCLC? Which immune checkpoint serves as the key regulator in anti-tumor responses in SCLC? How will immune responses change during the progress of SCLC? Is it possible that different major effector cells are involved in NSCLC and SCLC? Further studies are needed.

Conclusions

Immunocheckpoints play significant role in tumor immune escape with pathways closely interrelated to one another. Some SCLC patients have LAG-3-positive TILs. LAG-3 is prominently co-functioned with PD-1/PD-L1, and one of the meaningful predictions for their expression. Combination of LAG-3 and PD-1/PD-L1 inhibitors might become a promising immunotherapy for SCLC.

List Of Abbreviations

Lymphocyte activating gene-3 (LAG-3), non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), tumor-infiltrating lymphocytes (TILs)

Declarations

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This research was approved by the ethics committee of the Shanghai Pulmonary Hospital, Tongji University (No. K20-022). Written consent was given by all patients, and the experiment confirmed with the tenets of the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Liping Zhang preformed all IHC staining and checked the results. Yi Xu was responsible for all the statistic analysis, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledges

Not applicable

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.

2. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Akinyemiju TF, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2016: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol* 2018;4:1553-68.
3. van Meerbeeck JP, Fennell DA, De Ruyscher DK: Small-cell lung cancer. *Lancet* 378:1741-1755, 2011
4. Travis WD. Advances in neuroendocrine lung tumors. *Ann Oncol* 2010;21(Suppl 7):vii65-71.
5. Zakowski MF. Pathology of small cell carcinoma of the lung. *Semin Oncol* 2003;30:3–8.
6. Rudin CM, Giaccone G, Ismaila N. Treatment of Small-Cell Lung Cancer: American Society of Clinical Oncology Endorsement of the American College of Chest Physicians Guideline. *J Oncol Pract*. 2016 Jan;12(1):83-86. doi: 10.1200/JOP.2015.008201. PubMed PMID: 29424581.
7. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 2004;21:137-48.
8. Woo SR, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012;72:917–927.
9. Takaya S, Saito H, Ikeguchi M. Upregulation of immune checkpoint molecules, PD-1 and LAG-3, on CD4. and CD8. T cells after gastric cancer surgery. *Yonago Acta Med*. 2015;58:39–44.
10. He Y, Rozeboom L, Rivard CJ, et al. PD-1, PD-L1 Protein Expression in Non-Small Cell Lung Cancer and Their Relationship with Tumor-Infiltrating Lymphocytes. *Med Sci Monit*. 2017;23:1208-1216. Published 2017 Mar 9. doi:10.12659/msm.899909
11. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, et al. Safety and activity of anti- PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012; 366:2455-2465.
12. Horn L, Mansfield AS, Szczesna A, Havel L, Krzakowski M, Hochmair MJ, Huemer F, Losonczy G, Johnson ML, Nishio M, Reck M, Mok T, Lam S, Shames DS, Liu J, Ding B, Lopez-Chavez A, Kabbinavar F, Lin W, Sandler A, Liu SV; IMpower133 Study Group. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. *N Engl J Med*. 2018 Dec 6;379(23):2220-2229. doi: 10.1056/NEJMoa1809064. Epub 2018 Sep 25. PubMed PMID: 30280641.
13. Ragavan M, Das M. Systemic Therapy of Extensive Stage Small Cell Lung Cancer in the Era of Immunotherapy. *Curr Treat Options Oncol*. 2020;21(8):64. Published 2020 Jun 29. doi:10.1007/s11864-020-00762-8
14. Son B, Lee S, Youn H, Kim E, Kim W, Youn B. The role of tumor microenvironment in therapeutic resistance. *Oncotarget*. 2017; 8:3933-3945.
15. Zhao X, Subramanian S. Intrinsic Resistance of Solid Tumors to Immune Checkpoint Blockade Therapy. *Cancer Res*. 2017;77:817-822
16. He Y, Rivard CJ, Rozeboom L, Yu H, Ellison K, Kowalewski A, Zhou C, Hirsch FR. Lymphocyte-activation gene-3, an important immune checkpoint in cancer. *Cancer Sci*. 2016; 107:1193-1197.

17. Marconcini R, Spagnolo F, Stucci LS, Ribero S, Marra E, Rosa F, Picasso V, Di Guardo L, Cimminiello C, Cavalieri S, Orgiano L, Tanda E, Spano L, et al. Current status and perspectives in immunotherapy for metastatic melanoma. *Oncotarget*. 2018; 9:12452-12470.
18. Triebel F, Jitsukawa S, Baixeras E, Roman-Roman S, Genevee C, Viegas- Pequignot E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med* (1990) 171(5):1393–405. doi:10.1084/jem.171.5.1393
19. Lichtenegger FS, Rothe M, Schnorfeil FM, et al. Targeting LAG-3 and PD-1 to Enhance T Cell Activation by Antigen-Presenting Cells. *Front Immunol*. 2018;9:385. Published 2018 Feb 27. doi:10.3389/fimmu.2018.00385
20. He Y, Yu H, Rozeboom L, et al. LAG-3 Protein Expression in Non-Small Cell Lung Cancer and Its Relationship with PD-1/PD-L1 and Tumor-Infiltrating Lymphocytes. *J Thorac Oncol*. 2017;12(5):814-823. doi:10.1016/j.jtho.2017.01.019
21. Triebel F, Jitsukawa S, Baixeras E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med*. 1990;171:1393–1405.
22. Baixeras E, Huard B, Miossec C, Jitsukawa S, Martin M, Hercend T, et al. Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. *J Exp Med* (1992) 176(2):327–37. doi:10.1084/jem.176.2.327
23. Huard B, Prigent P, Tournier M, Bruniquel D, Triebel F. CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-IG fusion proteins. *Eur J Immunol* (1995) 25(9):2718–21. doi:10.1002/eji.1830250949
24. Andrews LP, Marciscano AE, Drake CG, et al. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev* 2017;276:80–96. [PubMed: 28258692]
25. Nguyen LT, Ohashi PS. Clinical blockade of PD1 and LAG3–potential mechanisms of action. *Nat Rev Immunol* 2015;15:45–56. [PubMed: 25534622]
26. Long L, Zhang X, Chen F, et al. The promising immune checkpoint LAG-3: from tumor microenvironment to cancer immunotherapy. *Genes Cancer*. 2018;9(5-6):176-189. doi:10.18632/genesandcancer.180
27. Blackburn SD, Shin H, Haining WN, et al. Coregulation of CD8+T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol*. 2009;10: 29–37.
28. Fougerey S, Brignone C, Triebel F. A soluble LAG-3 protein as an immunopotentiator for therapeutic vaccines: preclinical evaluation of IMP321. *Vaccine*. 2006;24: 5426–5433.
29. Santoiemma PP, Powell DJ Jr. Tumor infiltrating lymphocytes in ovarian cancer. *Cancer Biol Ther*. 2015; 16:807-820.
30. Xu Y, Wang L, Li W, et al. Killer immunoglobulin-like receptors/human leukocyte antigen class-I, a crucial immune pathway in cancer. *Ann Transl Med*. 2020;8(5):244. doi:10.21037/atm.2020.01.84
31. Andrews LP, Marciscano AE, Drake CG, et al. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev* 2017;276:80–96. [PubMed: 28258692]

32. Harris-Bookman S, Mathios D, Martin AM, et al. Expression of LAG-3 and efficacy of combination treatment with anti-LAG-3 and anti-PD-1 monoclonal antibodies in glioblastoma. *Int J Cancer*. 2018;143(12):3201-3208. doi:10.1002/ijc.31661
33. Datar I, Sanmamed MF, Wang J, et al. Expression Analysis and Significance of PD-1, LAG-3, and TIM-3 in Human Non-Small Cell Lung Cancer Using Spatially Resolved and Multiparametric Single-Cell Analysis. *Clin Cancer Res*. 2019;25(15):4663-4673. doi:10.1158/1078-0432.CCR-18-4142
34. Matsuzaki J, Gnjjatic S, Mhawech-Fauceglia P, Beck A, Miller A, Tsuji T, et al. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc Natl Acad Sci U S A*. 2010; 107:7875–80. [PubMed: 20385810]
35. Ascierto PA, Melero I, Bhatia S, Bono P, Sanborn RE, Lipson EJ, Callahan MK, Gajewski T, Gomez-Roca CA, Hodi FS, Curigliano G, Nyakas M, Preusser M, et al. Initial efficacy of anti-lymphocyte activation gene-3 (anti-LAG-3; BMS-986016) in combination with nivolumab (nivo) in pts with melanoma (MEL) previously treated with anti-PD-1/PD-L1 therapy. *J Clin Oncol*. 2017; 35:9520.
36. Ascierto PA, Bono P, Bhatia S, Melero I, Nyakas M, Svane I, Larkin J, Gomez-Roca CA, Schadendorf D, Dummer R, Marabelle A, Hoeller C, Maurer M, et al. Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in pts with melanoma. In: ESMO 2017 Congress, Madrid, Spain. *Genes & Cancer* 186
37. Sabari JK, Lok BH, Laird JH, Poirier JT, Rudin CM. Unravelling the biology of SCLC: implications for therapy. *Nat Rev Clin Oncol*. 2017;14(9):549-561. doi:10.1038/nrclinonc.2017.71
38. Yu H, et al. PD-L1 expression by two complementary diagnostic assays and mRNA in situ hybridization in small cell lung cancer. *J Thorac Oncol*. 2017; 12:110–120. [PubMed: 27639678]
39. Rivalland G, Walkiewicz M, Wright G, et al. Small cell lung cancer: the immune microenvironment and prognostic impact of checkpoint expression. *J Clin Oncol*. 2017;35:abstract 8569.
40. W. Wang, P. Hodgkinson, F. McLaren, et al., Small cell lung cancer tumour cells induce regulatory T lymphocytes, and patient survival correlates negatively with FOXP3+ cells in tumour infiltrate, *Int. J. Cancer* 131 (2012) E928–937.

Tables

Table 1. Patient Characteristics
(N=102)

Characteristic	n (%)
Sex	
Male	18 (17.6%)
Female	84 (82.4%)
Age, median, y	
<70	79 (77.5%)
≥70	23 (22.5%)
Smoking status	
Nonsmoker	58 (56.9%)
Smoker	44 (43.1%)
T stage	
1	40 (39.2%)
2	47 (46.1%)
3	13 (12.7%)
4	2 (2.0%)
N stage	
0	44 (43.1%)
1	23 (22.5%)
2	34 (33.3%)
3	1 (1.1%)
M stage	
0	98 (96.1%)
1	4 (3.9%)
Lung cancer stage	
I	38 (37.3%)
II	22 (21.5%)
III	42 (41.2%)
Chemotherapy	

Yes	54 (52.9%)
No	48 (47.1%)

Table 2. Relationships between Different Checkpoints

LAG-3 Expression on TILs			
Characteristic	Negative	Positive	p Value
PD-1 expression on TILs, n (%)			
Negative	47 (77.1%)	14 (22.9%)	0.006
Positive	15 (36.6%)	26 (63.4%)	
PD-L1 expression on tumor cells, n (%)			
Negative	60 (61.2%)	38 (38.8%)	0.365
Positive	2 (50.0%)	2 (50.0%)	
PD-L1 expression on TILs, n (%)			
Negative	49 (75.4%)	16 (24.6%)	0.001
Positive	13 (34.2%)	25 (65.8%)	

Table 3. Univariate and Multivariate Analysis for Prediction of PD-L1 Expression on TILs in SCLC Patients

Variables	Univariate			Multivariate		
	ORs	95% CI	p Value	ORs	95% CI	p Value
Age (<70y vs. ≥70y)	1.148	0.435-3.031	0.781			
Sex (female vs. male)	1.440	0.513-4.039	0.488			
Smoking status (nonsmoker vs. smoker)	0.760	0.339-1.707	0.507			
Stage (I–II vs. III)	0.056	0.980-5.424	2.306			
Chemotherapy (yes vs. no)	1.020	0.456-2.279	0.962			
LAG-3 (negative vs. positive)	0.159	0.066-0.386	0.000	0.161	0.063-0.412	0.000

Table 4. Univariate and Multivariate Analysis for Prediction of PD-1 Expression on TILs in SCLC Patients

Variables	Univariate			Multivariate		
	ORs	95% CI	p Value	ORs	95% CI	p Value
Age (<70y vs. ≥70y)	1.059	0.409-2.739	0.906			
Sex (female vs. male)	1.236	0.442-3.455	0.686			
Smoking status (nonsmoker vs. smoker)	1.326	0.593-2.964	0.492			
Stage (I–II vs. III)	2.339	1.010-5.414	0.047	3.102	1.147-8.390	0.026
Chemotherapy (yes vs. no)	0.412	0.181-0.935	0.034	0.339	0.129-0.895	0.029
LAG-3 (negative vs. positive)	0.172	0.072-0.411	0.000	0.193	0.078-0.480	0.000

Table 5. Univariate and Multivariate Analysis for RFS in All Patients

Variables	Univariate			Multivariate		
	ORs	95% CI	p Value	ORs	95% CI	p Value
Age (<70y vs. ≥70y)	1.770	0.997-3.143	0.051			
Sex (female vs. male)	1.676	0.757-3.707	0.203			
Smoking status (nonsmoker vs. smoker)	1.693	0.990-2.896	0.054			
Stage (I–II vs. III–IV)	2.111	1.243-3.586	0.006	1.885	1.102-3.223	0.021
Chemotherapy (yes vs. no)	0.770	0.454-1.305	0.331			
PD-1 on TILs (negative vs. positive)	0.981	0.959-1.004	0.104			
PD-L1 on tumor cells (negative vs. positive)	0.815	0.198-3.347	0.776			
PD-L1 on TILs (negative vs. positive)	0.417	0.223-0.779	0.006			
LAG-3 on TILs (negative vs. positive)	0.612	0.348-1.076	0.088			
CD3 (negative vs. positive)	0.480	0.281-0.820	0.007			
CD4 (negative vs. positive)	0.450	0.245-0.825	0.010			
CD8 (negative vs. positive)	0.400	0.206-0.776	0.007			
FOXP3 (negative vs. positive)	0.376	0.194-0.730	0.004			

Figures

Figure 1

Lymphocyte -activating gene 3 (LAG-3) and relapse free survival (RFS) in SCLC