Review Article

The landscape of immune checkpoints expression in non-small cell lung cancer: a narrative review

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Abstract: With the increasing clinical potential of tumor immunotherapy, more and more clinical trials are undergoing with immune checkpoint inhibitors (ICIs). Immune checkpoints (ICs) have been identified as crucial regulators of the immune response and have improved ICIs-inhibitor therapeutic strategies. The most important ICs in lung cancer include programmed cell death-1 (PD-1), programmed cell death ligand-1 (PD-L1), lymphocyte activation gene-3 (LAG-3), major histocompatibility complex class II (MHC II), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), and Galectin-9 (GAL-9), OX-40, OX40L. However, the expression and prognostic value of these ICs are still controversial. Among them, high expression of PD-L1 on tumor cells (>50%) predicts a better therapeutic effect of anti-PD-1 monoclonal antibody compared to patients with low PD-L1 expression. However, only 20–30% of non-small cell lung cancer (NSCLC) patients seem to get benefit from immunotherapy. In order to improve the immunotherapy outcomes, more and more attention is paid to combination immunotherapy. Analyzing the co-expression of ICs can give us a more comprehensive basis for combination immunotherapy. This review article summarized our comprehensive expression of ICs based on our previous research, and analyzed their correlation with prognosis in NSCLC patients. We also provided suggestions for potentially personalized combination immunotherapy in NSCLC.

Keywords: Immune checkpoints (ICs); prognosis; non-small cell lung cancer (NSCLC); combination immunotherapy

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Introduction

There were 2.1 million new lung cancer cases worldwide, accounting for 11.6% of all new cancer cases. With 1.8 million deaths, accounting for 18.4% of all cancer deaths, the incidence of lung cancer ranked first among all cancers in most countries and continues to rise (1). The five-year survival rate for lung cancer is 4–17%, and about 80% of lung cancer patients are non-small cell lung cancer (NSCLC) (2). Nowadays, surgical treatment is still the primary method for early-stage lung cancer (3). Most patients are diagnosed too late for being surgical candidates with a relatively lower chance of long-term survival or cure. Chemotherapy has been an important role in the treatment of advanced NSCLC, but its five-year survival rate is only 5% (4). Targeted therapy has a good therapeutic outcome,

but drug resistance is unavoidable in most patients and many patients do not have proper gene mutations for specific targeted therapy. Cancer immunotherapy has been a promising therapy nowadays (5).

Cancer immunotherapy depends on activating T cells from inhibitory status and functional exhaustion to eliminate tumor cells, which could be promoted by some tumor-infiltrating lymphocytes (TILs). However, a variety of lymphocytes, as a kind of infiltrating immune cells in cancers, could attack cancer cells or promote the immune escape of cancer cells (6,7). Additionally, abnormal expression of immune checkpoint (ICs) molecules is one of the mechanisms of tumor immune escape. The basic principle of tumor immunotherapy is to apply immune checkpoint inhibitors (ICIs) to block the transmission of inhibitory signals and thereby induce an anti-tumor effect. Some ICs of tumors will inhibit T cell proliferation or anti-tumor activity, so the expression of the ICs will be directly associated with tumor immune escape. As recently reported in the literature, tumor cell-intrinsic programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) could suppress the canonical signaling pathways, regulate tumor growth, and mediate the resistance to the treatment with anti-PD-1/PD-L1 antibodies (8). Therefore, the expression of ICs and TILs is crucial to cancer immunotherapy.

PD-1 and PD-L1 inhibitors have shown encouraging results in NSCLC and other cancers. They have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of advanced NSCLC (9-13). Lymphocyte activation gene-3 (LAG-3), as a co-inhibitory molecule of PD-1, has been proved to play a role in immune escape in various tumors (14,15). Since the activation of antigen-specific CD4+ T cells requires the presence of major histocompatibility complex class II (MHC II) antigens, MHC II is important in anti-tumor immunity (16,17). T cell immunoglobulin and mucin-domain containing-3 (TIM-3) is considered to be a negative regulator of CD4+ T cells and CD8+ T cells (18). Galectin-9 (GAL-9) also plays the role of manipulating the T effector immune cells and predicting the survival of patients with LAG-3 and TIM-3 (19,20). OX40 (CD134) has been shown to rebuild and enforce the weakened immune system and combat malignant cells (21,22).

Many studies showed that the expression of ICs conveyed prognostic and predictive information (2,6,23). Identifying patients who might get the benefits from immunotherapy will protect patients from unnecessary treatments and side effects. However, the accurate prognostic biomarkers of these ICs in NSCLC remain controversial. In this study, we reviewed several important ICs in NSCLC from our published paper (9,14,16,17,20-22,24-26), including the frequency and co-expression of ICs. The goal of this review is to give an overall demonstration of the expression of ICs and the correlation with prognosis in NSCLC and to suggest some potentially personalized combination immunotherapies in NSCLC.

We present the study in accordance with the Narrative Review Checklist (Available at http://dx.doi.org/10.21037/tlcr-20-1019).

Correlation between ICs and clinical pathological factors in NSCLC

Immunological factors are not only statistically related to some clinically pathological factors, but also functionally interact with each other in NSCLC. In this review, the reported immunological factors are potentially important for the immune regulation in NSCLC. ICs correlate with other ICs. LAG-3 has been reported as a clinically important immunological factor in various kinds of tumors (27-29). In breast cancer, Saleh *et al.* (30) not only found that tumor cells could affect the expression of LAG-3, PD-1, and TIM-3 but also illustrated that the status of PD-1 and PD-L1 further influenced the expression of TIM-3 and LAG-3 on TILs. Datar *et al.* (31) found that the distinct tissue/cell distribution of PD-1, LAG-3, and TIM-3, and their interaction results, which concluded that their immune evasion pathways were independent. Both tumors and TILs have an expression of MHC II (32-34). In gastric carcinomas, Ma *et al.* (35) found that the expression of MHC II was related to the differentiation of tumor and TILs. In lung cancer, the impairment of MHC-DR expression was reported as one of the major reasons for immunosuppression (36). GAL-9, a member of the beta-galactoside-binding animal lectin family, participates in various cellular biological events, while its function as the immunological factor was paradoxical. In hepatocellular carcinoma (HCC), Fujita *et al.* (37) found that the expression of GAL-9 led to the apoptosis of HCC cells, which was also reported in other malignancies (38,39). However, GAL-9 was found to be suppressed by the autoimmune response as well (40). OX40 was recognized as the potential target for cancer immunotherapy (41). In advanced colorectal cancer, the expression of OX40 was reported to be related to the blood levels of other biomarkers, including PD-L1 (42). However, data on the expression of these ICs and their correlation with other clinically pathological factors in NSCLC are incomplete. We first reviewed the characteristics of potential immune

biomarkers of NSCLC patients, which including PD-1, PD-L1, LAG-3, MHC II, TIM-3, GAL-9, OX40, and OX40L (In our published paper, 3 patients were not stained with GAL-9 on tumor cells or TILs (20). We re-stained the GAL-9 in these 3 patients and got a negative result.

It was reported that the expression of OX40 on TILs was significantly correlated to smoking status, lung cancer pathology, and percentage of TILs (21). Besides, lung cancer pathology was significantly correlated to the expression of LAG-3 on TILs (14) and MHC II on tumors (16). Likewise, the lung cancer stage was correlated to the expression of OX40L on tumors (21), PD-L1 on tumors (9), MHC II on TILs (16). GAL-9 on tumors was the only ICs that had a significant correlation with the grade (20). However, there was no significant correlation among clinical-pathological factors and the expression of OX40 in tumors, PD-L1 on tumors, GAL-9 on TILs, TIM-3 on tumors, and TIM-3 on TILs (9,20,21,26). *Table 1* provided the detailed information of various ICs in NSCLC and their correlation with clinic pathological factors and other ICs, indicating that the smoking status, lung cancer pathology, stage, grade, metastasis, and TILs percentage were worthy of clinical attention as the complicated relationship between these factors and the status of ICs. Having a better understanding of the relationship between clinical factors and ICs can help us have more information on ICIs treatment and predict the disease progression of NSCLC patients.

The landscape of ICs in NSCLC

The innovative uses of the Upset and Venn plot help comprehend the relationship between these biomarkers clearly and comprehensively (*Figure 1*). Our review demonstrated that the positive status of different ICs could be variable in NSCLC patients.

Comparative analysis was carried out with Bioinformatics and Evolutionary Genomics tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) and TBtools (43). The Upset plot showed the distribution of each IC in NSCLC. The bar graph above showed the number of ICs contained in each type of ICs group. The yellow bar graph at the bottom left showed the number of ICs contained in each type of ICs. *Figure 1* demonstrated the co-expression of ICs in NSCLC and TILs in upset plot or Venn plot. According to the Upset plot and Venn plot below, a substantial proportion (76.5%) of patients had a unique ICs combination. Unlike the mutually exclusive NSCLC driver genes, these figures indicated that co-expression of ICs was widespread, suggesting that immunotherapy combination may have a promising future. *Table 2* and *Figure 2* showed the distribution of multiple ICs positive patients. According to the pie chart, the ICs were not mutually exclusive, most patients (82.0%) have 1 to 6 positive ICs, and only 7 (5.0%) patients had no ICs expression. OX40L on TILs and OX40 on tumors were the top two high expressions in ICs and patients usually expressed them simultaneously, which might suggest that the combination therapy using these two target drugs may benefit patients to a large extent.

In this pie chart (*Figure 2*), it showed the number of positive ICs in patients, consisting of 11 degrees, reflecting directly ICs multiple positive distributions.

Independent prognostic factors for overall survival (OS) and relapse-free survival (RFS) in NSCLC patients

The ICs might be related to the prognosis of cancer. In chronic lymphocytic leukemia (CLL), Kotaskova *et al*. (44) found that the higher expression of LAG-3 was correlated with shorter treatment-free survival, which indicated that LAG-3 was one of the novel prognostic markers in CLL. In transplantable tumor mouse models, Woo *et al*. (45) found that the combination therapy of anti-LAG-3 antibody and anti-PD-1 antibody dramatically led to a better prognosis. MHC II was also reported as one of the independent prognostic factors for different types of cancers (34,35,46). In renal cell carcinomas (RCC), Brasanac *et al*. (47) showed that the activation of TILs was affected by the expression of MHC II antigen, thus leading to the different prognosis of cancer. In esophageal squamous cell carcinoma, the positive expression of MHC-DR was regarded as the biomarker for tumor invasion, while the negative expression of it referred to the promoter of tumor escape (35,48,49). GAL-9 and TIM-3 were also considered as a prognostic indicator for various cancers (50,51). In the matter of OX40 and OX40L, the expression of OX40 on TILs was shown to be related to the survival of cancer patients, such as hepatocellular carcinoma and acute myeloid leukemia (42,52,53). However, a consensus was not reached for the predictive ability of OX40 and OX40L (54).

The survival curves and outcomes of fractional variables were analyzed separately in our previous publications (9,14,16,20,21,26). However, for the sake of roundly comparing OS differences among NSCLC patients in different conditions, we summed up and corrected the previous outcomes, and supplemented the missing data utilizing the Kaplan-Meier method (K-M method) (Tables S1,S2). OX40L expression

Table 1 Relationship between various immune characteristic biomarkers and clinical data (immunohistochemistry for all samples from the Medical University of Gdansk)

Variable	Age, n (%)		Gender, n (%)		Smoker, n (%)		stage, n (%)		Pathology, n (%)		Grade, n (%)		TILs, n (%)		Metastasis, n (%)		Total (n, %)
	<70	≥70	Female	Male	NS	Smoker	I–II	III–IV	Non-AC	AC	G1	G2-3	<30%	≥30%	–	+	
OX40-tumors (20)																	
–	94 (89.5)	31 (91.2)	26 (86.7)	99 (90.8)	5 (83.3)	120 (90.2)	83 (89.2)	42 (91.3)	88 (88.9)	37(92.5)	33(94.3)	92(88.5)	56(88.9)	69(90.8)	118(89.4)	7(100.0)	125(89.9)
+	11 (10.5)	3 (8.8)	4 (13.3)	10 (9.2)	1 (16.7)	13 (9.8)	10 (10.8)	4 (8.7)	11 (11.1)	3(7.5)	2(5.7)	12(11.5)	7(11.1)	7(9.2)	14(10.6)	0(0.0)	14(10.1)
P	0.781		0.743		1.000		0.936		0.742		0.506		0.711		1.000		
OX40-TILs (20)																	
–	56 (53.3)	12 (35.3)	13 (43.3)	55 (50.5)	0 (0.0)	68 (51.1)	43 (46.2)	25 (54.3)	43 (74.1)	25(30.9)	15(42.9)	53(51.0)	41(65.1)	27(35.5)	63(47.7)	5(71.4)	68(48.9)
+	49 (46.7)	22 (64.7)	17 (56.7)	54 (49.5)	6 (100.0)	65 (48.9)	50 (53.8)	21 (45.7)	15 (25.9)	56(69.1)	20(57.1)	51(49.0)	22(34.9)	49(64.5)	69(52.3)	2(28.6)	71(51.1)
P	0.067		0.489		0.028*		0.368		0.042*		0.407		0.001*		0.404		
OX40L-tumors (20)																	
–	78 (74.3)	25 (73.5)	24 (80.0)	79 (72.5)	3 (50.0)	100 (75.2)	75 (80.6)	28 (60.9)	70 (70.7)	33(82.5)	22(62.9)	81(77.9)	47(74.6)	56(73.7)	99(75.0)	4(57.1)	103(74.1)
+	27 (25.8)	9 (26.5)	6 (20.0)	30 (12.5)	3 (50.0)	33 (24.8)	18 (19.4)	18 (39.1)	29 (29.3)	7(17.5)	13(37.1)	23(22.1)	16(25.4)	20(26.3)	33(25.0)	3(42.9)	36(25.9)
P	0.930		0.405		0.180		0.012*		0.151		0.079		0.902		0.375		
OX40L-TILs (20)																	
–	29 (27.6)	8 (23.5)	11 (36.7)	26 (23.8)	0 (0.0)	37 (27.8)	23 (24.7)	14 (30.4)	24 (24.2)	13(32.5)	9(25.7)	28(26.9)	18(28.6)	19(25.0)	0(0.0)	37(84.1)	37(26.6)
+	76 (72.4)	26 (76.5)	19 (63.3)	83 (76.2)	6 (100.0)	96 (72.2)	70 (75.3)	32 (69.6)	75 (75.8)	27(67.5)	26(74.3)	76(73.1)	45(71.4)	57(75.0)	95(100.0)	7(15.9)	102(73.4)
P	0.639		0.160		0.342		0.474		0.319		0.889		0.758		<0.001*		
PD-1-TILs (8)																	
–	62 (59.0)	17 (50.0)	14 (46.7)	65 (59.6)	4 (66.7)	75 (56.4)	53 (57.0)	26 (56.5)	54 (54.4)	25(62.5)	18(51.4)	61(58.7)	52(82.5)	27(35.5)	74(56.1)	5(71.4)	79(56.8)
+	43 (41.0)	17 (50.0)	16 (53.3)	44 (40.4)	2 (33.3)	58 (43.6)	40 (43.0)	20 (43.5)	45 (45.5)	15(37.5)	17(48.6)	43(41.3)	11(17.5)	49(64.5)	58(43.9)	2(28.6)	60(43.2)
P	0.356		0.207		0.622		0.959		0.392		0.802		<0.001*		0.683		
PD-L1-tumors (8)																	
–	86 (81.9)	28 (82.4)	23 (76.7)	91 (83.5)	4 (66.7)	110 (82.7)	78 (83.9)	36 (78.3)	79 (79.8)	35(87.5)	25(71.4)	89(85.6)	52(82.5)	62(81.6)	108(81.8)	6(85.7)	114(82.0)
+	19 (18.1)	6 (17.6)	7 (23.3)	18 (16.5)	2 (33.3)	23 (17.3)	15 (16.1)	10 (21.7)	20 (20.2)	5(12.5)	10(28.6)	15(14.4)	11(17.5)	14(18.4)	24(18.2)	1(14.3)	25(18.0)
P	0.953		0.392		0.330		0.419		0.289		0.895		0.883		1.000		
PD-L1-TILs (8)																	
+	68 (64.8)	21 (61.8)	20 (66.7)	69 (63.3)	4 (66.7)	85 (63.9)	54 (58.1)	35 (76.1)	60 (60.6)	29(72.5)	23(65.7)	66(63.5)	52(82.5)	37(48.7)	84(63.6)	5(71.4)	89(64.0)
–	37 (35.2)	13 (38.2)	10 (33.3)	40 (36.7)	2 (33.3)	48 (36.1)	39 (41.9)	11 (23.9)	39 (39.4)	11(27.5)	12(34.3)	38(36.5)	11(17.5)	39(51.3)	48(36.4)	2(28.6)	50(36.0)

Table 1 *(continued)*

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Variable	Age, n (%)		Gender, n (%)		Smoker, n (%)		stage, n (%)		Pathology, n (%)		Grade, n (%)		TILs, n (%)		Metastasis, n (%)		Total (n, %)
	<70	≥70	Female	Male	NS	Smoker	I–II	III–IV	Non-AC	AC	G1	G2-3	<30%	≥30%	–	+	
P	0.752		0.734		1.000		0.037*		0.186		0.810		<0.001*		0.988		
GAL-9-tumors (19)																	
–	62 (59.0)	22 (64.7)	21 (70.0)	63 (57.8)	3 (50.0)	81 (60.9)	51 (54.8)	33 (71.7)	58 (58.6)	26(65.0)	249(68.6)	40(47.6)	36(57.1)	48(63.2)	79(59.8)	5(71.4)	84(60.4)
+	43 (41.0)	12 (35.3)	9 (30.0)	46 (42.2)	3 (50.0)	52 (39.1)	42 (45.2)	13 (28.3)	41 (41.4)	14(35.0)	11(31.4)	44(52.4)	27(42.9)	28(36.8)	53(40.2)	2(28.6)	55(39.6)
P	0.558		0.226		0.681		0.055		0.484		0.037*		0.470		0.831		
GAL-9-TILs (19)																	
–	99 (94.3)	31 (91.2)	28 (93.3)	102 (93.6)	5 (83.3)	125 (94.0)	87 (93.5)	43 (93.5)	92 (92.9)	38(95.0)	30(85.7)	100(96.2)	61(96.8)	69(90.8)	123(93.2)	7(100.0)	130(93.5)
+	6 (5.7)	3 (8.8)	2 (6.7)	7 (6.4)	1 (16.7)	8 (6.0)	6 (6.5)	3 (6.5)	7 (7.1)	2(5.0)	5(14.3)	4(3.8)	2(3.2)	7(9.2)	9(6.8)	0(0.0)	9(6.5%)
P	0.811		1.000		0.850		1.000		0.945		0.076		0.274		1.000		
LAG-3-TILs (13)																	
–	77 (73.3)	26 (76.5)	21 (70.0)	82 (75.2)	5 (83.3)	98 (73.7)	71 (76.3)	32 (69.6)	68 (68.7)	35(87.5)	26(74.3)	77(74.0)	73(79.4)	53(69.7)	97(73.5)	6(85.7)	103(74.1)
+	28 (26.7)	8 (23.5)	9 (30.0)	27 (24.8)	1 (16.7)	35 (26.3)	22 (23.7)	14 (30.4)	31 (31.3)	5(12.5)	9(25.7)	27(26.0)	13(20.6)	23(30.3)	35(26.5)	1(14.3)	36(25.9)
P	0.717		0.563		0.959		0.391		0.031*		0.977		0.197		0.782		
MHC II-tumors (15)																	
–	73 (69.5)	24 (70.6)	17 (56.7)	80 (73.4)	2 (33.3)	95 (71.4)	60 (64.5)	37 (80.4)	77 (77.8)	20(50.0)	13(81.3)	89(85.6)	47(74.6)	50(65.8)	91(68.9)	6(85.7)	97(69.8)
+	32 (30.5)	10 (29.4)	13 (43.3)	29 (26.6)	4 (66.7)	38 (28.6)	33 (35.5)	9 (19.6)	22 (22.2)	20(50.0)	3(18.7)	15(14.4)	16(25.4)	26(34.2)	41(31.1)	1(14.3)	42(30.2)
P	1.000		0.115		0.068		0.077		0.002*		0.707		0.273		0.603		
MHC II-TILs (15)																	
–	65 (61.9)	19 (55.9)	15 (50.0)	69 (63.3)	2 (33.3)	82 (61.7)	50 (53.8)	34 (73.9)	61 (61.6)	23(57.5)	63(86.3)	39(83.0)	37(58.7)	47(61.8)	77(58.3)	7(100.0)	84(60.4)
+	40 (38.1)	15 (44.1)	15 (50.0)	40 (36.7)	4 (66.7)	51 (38.3)	43 (46.2)	12 (26.1)	28 (38.4)	17(42.5)	10(13.7)	8(17.0)	26(41.3)	29(38.2)	55(41.7)	0(0.0)	55(39.6)
P	0.551		0.210		0.213		0.027*		0.704		0.612		0.730		0.028*		

Table 1 *(continued)*

Table 1 *(continued)*

Variable	Age, n (%)		Gender, n (%)		Smoker, n (%)		stage, n (%)		Pathology, n (%)		Grade, n (%)		TILs, n (%)		Metastasis, n (%)		Total
	<70	≥70	Female	Male	NS	Smoker	I–II	III–IV	Non-AC	AC	G1	G2-3	<30%	≥30%	–	+	(n, %)
TIM-3-tumors (25)																	
–	100 (95.2)	30 (88.2)	29 (96.7)	101 (92.7)	5 (83.3)	125 (94.0)	89 (95.7)	41 (8 9.1)	93 (93.9)	37(92.5)	16(100.0)	97(9 3.3)	56(88.9)	74(97.4)	125(94.7)	5(71.4)	137(98.6)
+	5 (4.8)	4 (11.8)	1 (3.3)	8 (7.3)	1 (16.7)	8 (6.0)	4 (4.3)	5 (10.9)	6 (6.1)	3(7.5)	0(0.0)	7(6.7)	7(11.1)	2(2.6)	7 (5.3)	2(28.6)	2(1.4)
P	0.222		0.684		0.336		0.157		0.717		0.592		0.079		0.066		
TIM-3-TILs (25)																	
–	99 (94.3)	29 (85.3)	28 (93.3)	100 (91.7)	5 (83.3)	123 (92.5)	87 (93.5)	41 (89.1)	91 (91.9)	37(92.5)	14(87.5)	98(94.2)	57(90.5)	71(93.4)	122(92.4)	6(85.7)	128(92.1)
+	6 (5.7)	5 (14.7)	2 (6.7)	9 (8.3)	1 (16.7)	10 (7.5)	6 (6.5)	5 (10.9)	8 (8.1)	3(7.5)	2(12.5)	6(5.8)	6(9.5)	5(6.6)	10(7.6)	1(14.3)	11(7.9)
P	0.137		1.000		0396		0.505		1.000		0.289		0.546		0.446		

OX40, Tumor necrosis factor receptor superfamily member 4; OX40L, OX40 ligand; PD-1, program death-1; PD-L1, program death-ligand 1; GAL-9, Galectin-9; LAG-3, Lymphocyte-activation gene-3; MHC II, Major histocompatibility complex class II; NS, non smoker; TIM-3, T-cell immunoglobulin and mucin domain-3; TILs, tumor-infiltrating lymphocytes; AC, adenocarcinoma; –, Negative; +. Positive; *, Statistically significant P values.

on tumors (21), GAL-9 expression on tumors (20), LAG-3 expression on TILs (14), MHC II expression on TILs (16), TIM-3 expression on TILs (26), gender, lung cancer stage, grade, and metastasis were significant independent prognostic factors for OS. Also, OX40 expression on tumors (21), MHC II expression on tumors (16), and pathology were also considered as the potential prognostic factors for OS.

Detailed information about different immunological factors as well as clinical characteristics on RFS was summarized and revised. GAL-9 expression on TILs (20), LAG-3 expression on TILs (14), MHC II expression on TILs (16), TIM-3 expression on TILs (26), lung cancer stage, grade, and metastasis were significant independent prognostic factors for RFS. In addition, OX40 expression on tumors (21), OX40L expression on tumors (21), OX40L expression on TILs (21), PD-L1 expression on tumors (9), GAL-9 expression on tumors (20), gender, and pathology were also considered as the potential prognostic factors for RFS.

Conclusions

Current guidelines emphasize the importance of accurate NSCLC sub-classifications for personalized precision medicine. Immunotherapy has a good result in lung cancer patients, and biomarkers are urgently needed to accurately sub-classify lung cancer and monitor its response.

Our review demonstrated the expression of 14 ICs on TILs and tumor cells in NSCLC and analyzed the correlations among different ICs. We also analyzed the correlation between ICs and prognosis. We found that the majority of patients were positive for multiple ICs expression, which provides a research basis for the development of bispecific and multi-targeted antibodies. And the predictive value of different ICs for prognosis provides the basis for stratified precision therapy.

This landscape of ICs in NSCLC could develop promising strategies for screening for suitable patients and conferring significant survival benefits for patients. In the future, we will continue to explore the role of more targets in different pathological types of cancer to provide theoretical support for combined immunotherapy.

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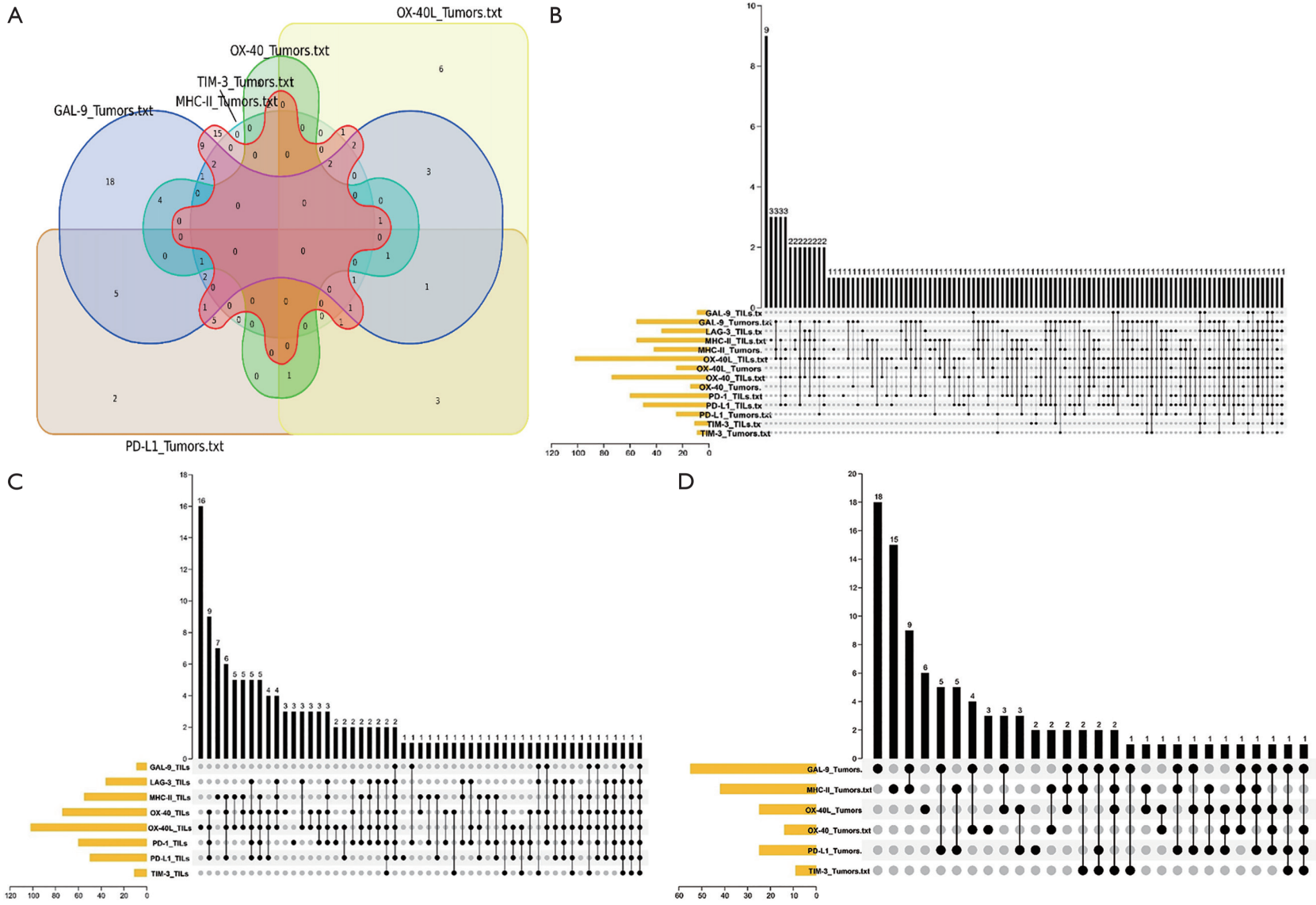

Figure 1 The landscape of Immune Checkpoints expression in non-small cell lung cancer (NSCLC). (A) The landscape of immune checkpoints in non-small cell lung cancer. In the Venn diagram, overlapping sections indicate multiple positive expressions of the same sample, and the number indicated identifies the number of samples in that section. (B) Upset plot displaying the co-expression of immune checkpoints (ICs) in NSCLC and tumor-infiltrating lymphocytes (TILs). In the Upset plot, the horizontal bar on the left shows the number of positives for each ICs, the black bead on the bottom right shows the co-expression of positives for the corresponding ICs, and the vertical bar on the top right shows the number of samples for that combination of ICs. (C) Upset plot displaying co-expression of ICs in TILs. In TILs, OX40L and OX40L were expressed highly. (D) Upset plot demonstrating co-expression of ICs in NSCLC tumor cells, with GAL-9 the highest expression.

Table 2 Distribution of multiple combinations of immune checkpoints (ICs) positive patients

Number of positive ICs	Number of patients	Percent
0	7	5.04%
1+	16	11.51%
2+	15	10.79%
3+	18	12.95%
4+	24	17.27%
5+	23	16.55%
6+	18	12.95%
7+	8	5.76%
8+	2	1.44%
9+	4	2.88%
10+	4	2.88%
Total	139	100%

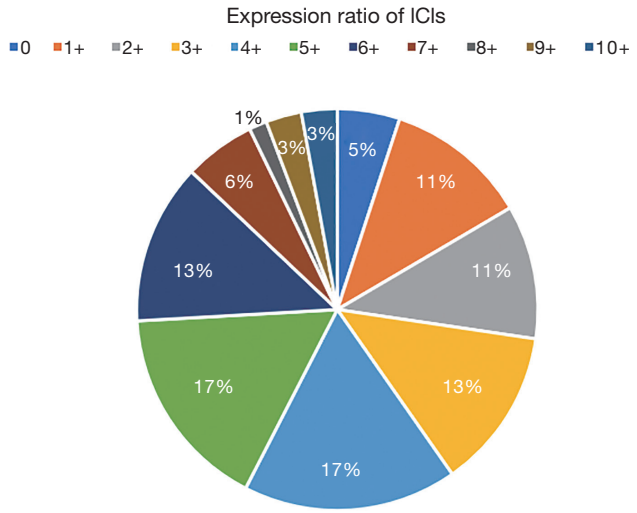

Figure 2 Distribution of multiple combinations of immune checkpoints (ICs) positive patients.

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Footnote

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