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Tumor and Tumor-Associated Macrophage PD-L1 Expression is Associated with Adjuvant Chemotherapy Benefit in Lung Adenocarcinoma

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Abstract

Introduction: Patients with stage II-III lung adenocarcinoma are treated with adjuvant chemotherapy (ACT) to target the premetastatic niche that persists after curative-intent resection. We hypothesized that the premetastatic niche is a scion of resected lung tumor microenvironment (TME) and that analysis of TME can stratify survival benefit from ACT.

Methods: Using tumor and tumoral stroma from 475 treatment-naive patients with stage II-III lung adenocarcinoma, we constructed a tissue microarray and performed multiplex immunofluorescent staining for immune markers (programmed death ligand-1 [PD-L1], tumor-associated macrophages [TAMs], and myeloid-derived suppressor cells [MDSCs]), and derived myeloid-lymphoid ratio (MLR). The association between immune markers and survival was assessed using Cox models adjusted for pathologic stage.

Results: Patients with high PD-L1 expression on TAMs or tumor cells in resected tumors had improved survival with ACT (TAMs: hazard ratio [HR], 1.79; 95% CI, 1.12–2.85; Tumor cells: HR, 3.02; 95% CI, 1.69–5.40). Among patients with high PD-L1 expression on TAMs alone or TAMs and tumor cells, ACT survival benefit is pronounced with high MLR (TAMs: HR, 3.87; 95% CI, 1.79–8.37; TAMs and tumor cells: HR, 2.19; 95% CI, 1.02–4.71) or with high stromal MDSC ratio (TAMs: HR, 2.53; 95% CI, 1.29–4.96; TAMs and tumor cells: HR, 3.21; 95% CI, 1.23–8.35). Patients with low/no PD-L1 expression on TAMs or tumor cells had no survival benefit from ACT.

Conclusions: Our observation that PD-L1 expression on TAMs or tumor cells is associated with improved survival with adjuvant chemotherapy provides rationale for prospective investigation and developing chemoimmunotherapy strategies for lung adenocarcinoma patients.

Keywords

NSCLC; Tumor immune microenvironment; Pre-metastatic niche; MDSCs; Myeloid-Lymphoid ratio

INTRODUCTION[1]

Postresection recurrence of early-stage lung adenocarcinomas is a considerable clinical challenge.[1,2] Adjuvant chemotherapy (ACT), administered as the standard of care for

[1]Abbreviations: ACT, adjuvant chemotherapy; COL, colloid carcinoma; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 second; HR, hazard ratio; ICI, immune checkpoint inhibitor; IMA, invasive mucinous adenocarcinoma; LC-CID, lung cancer cumulative incidence of death; LVI, lymphovascular invasion; MDSCs, myeloid-derived suppressor cells; MLR, myeloid-lymphoid ratio; NSCLC, non-small cell lung cancer; OS, overall survival; p-stage, pathologic stage; PD-L1, programmed death ligand-1; SHRs, sub-distribution hazard ratios;

patients with resected stage II–III lung adenocarcinoma, is associated with a 5-year overall survival (OS) benefit of 4%–15%.[3–5] With the exception of pathologic stage (p-stage), there are no known predictors of OS benefit from ACT.[6] The beneficial effect of ACT is thought to be derived from targeting the premetastatic niche, which comprises tumor cells and associated immunosuppressive cells that persists even after resection.[7,8]

We and others have shown that tumor immune microenvironment (TME) factors predict recurrence in early-stage lung adenocarcinomas.[9] In advanced lung adenocarcinomas, TME factors are associated with therapeutic benefit from immune checkpoint inhibitor (ICI) agents, specifically programmed death ligand-1 (PD-L1) expression on tumor-associated macrophages (TAMs) and tumor cells.[10–13] Owing to the known immunomodulatory effects of chemotherapy,[8] coupled with the increasing number of investigations of combination chemotherapy and ICI agents in non-small cell lung cancer (NSCLC),[14,15] the TME factors associated with benefit from ACT are of high interest. The Lung Adjuvant Cisplatin Evaluation Biomarker (LACE-Bio) collaborative group investigated tumor and immune cell PD-L1 expression in early-stage NSCLC and concluded that neither tumor nor immune cell PD-L1 expression is predictive of benefit from ACT.[16]

Whereas tumor and/or immune cell PD-L1 expression may indicate the activity of tumor infiltrating lymphocytes, tumor immunity is a balance of effector and suppressor immune responses.[17] The immunomodulatory effects of chemotherapy extend beyond the effector responses that result from the immunogenicity of cancer cell cytolysis.[8] Chemotherapeutic agents decrease immunosuppressive cells such as TAMs[18] and myeloid-derived superior cells (MDSCs), a type of immature myeloid cell recruited from bone marrow.[19] MDSCs play a substantial role in the formation of tumor metastasis, effecting OS in murine models and clinical studies.[20] In addition, immune cell ratios such as the myeloid-lymphoid ratio (MLR) in the TME and the neutrophil-lymphocyte ratio in peripheral blood have shown to be of prognostic value in solid tumors, including NSCLC.[21,22] However, the interrelation between PD-L1 expression on tumor cells and TAMs and MLR and MDSC ratio, and their association with OS benefit from chemotherapy, is not clear.

We hypothesized that the premetastatic niche immune microenvironment is a scion of the resected lung adenocarcinoma TME and that analysis of PD-L1 expression on TAMs and tumor cells (metrics of effector immune response) and MLR and MDSC ratio (metrics of suppressor immune response) in the lung adenocarcinoma TME can predict OS benefit from ACT.

MATERIALS AND METHODS

Patients

This study was approved by the institutional review board at Memorial Sloan Kettering Cancer Center. From our prospectively maintained database, we identified 1211 patients who had undergone surgical resection for p-stage II–III solitary primary lung

STAS, spread through air spaces; SUVmax, maximum standardized uptake value; TAMs, tumor-associated macrophages; TMA, tissue microarray; TME, tumor microenvironment

adenocarcinoma from January 2000 to December 2012. Exclusion criteria were receipt of induction therapy, lung cancer within 2 years, concurrent non–lung cancer disease progression, wedge resection, and positive surgical margin (see Figure, Supplemental Data 1, CONSORT diagram). No patients in ACT cohort received adjuvant radiation or ICI therapy.

Histologic Examination and Tissue Microarray (TMA) Construction

Tumors were classified according to the 8th edition International Association for the Study of Lung Cancer TNM classification of lung cancer.[23] Histologic examination of hematoxylin and eosin (H&E)–stained tumor slides was performed by a pathologist; tumor and stromal regions were marked. Six cores from the two predominant histologic subtypes and three from predominant stromal regions marked on the H&E-stained slides were identified on the corresponding formalin-fixed, paraffin-embedded tumor blocks, and cylindrical 0.6-mm tissue cores were arrayed onto a recipient block to construct a tissue microarray (TMA) (see Figure, Supplemental Data 2A).[9]

Multiplex Immunofluorescence Staining

Multiplexed staining of four consecutive sections of 4-μm thickness from each TMA were stained with four panels of antibodies (see Figure, Supplemental Data 2B) using the Opal 7-plex fIHC kit (Akoya Biosciences, Marlborough, MA). Stained slides were scanned, and high-power images of individual cores were captured using the Vectra 3.0 multispectral imaging system. Quantitative assessment of cell markers was performed using inForm software version 2.2.1. Cell segmentation and phenotyping algorithms were reviewed and confirmed by the study pathologists.

Statistical Analysis

Patient characteristics were summarized as frequency (percentage) and median (interquartile range) and compared across groups using the Wilcoxon rank-sum test for continuous variables and Fisher’s exact test for categorical variables. Distributions of cell counts were compared across the four groups using the Kruskal-Wallis test.[24] If results of the Kruskal-Wallis test were significant, secondary analyses were performed between pairs of groups using Dunn’s test. Dunn’s test is appropriate for groups with unequal numbers of observations. Comparisons of cell count distributions by ACT status within subsets defined by group and cell types were conducted using the Wilcoxon rank-sum test. False-discovery rate corrections were applied to address multiple testing.

The primary outcome of interest was OS (duration from surgery to death). Patients were otherwise censored at the last follow-up. OS was estimated using the Kaplan-Meier approach and compared between groups using log-rank tests. Relationships between groups and OS were quantified using Cox proportional hazards analyses, stratified by p-stage where appropriate.[25] Proportionality assumptions were assessed using Schoenfeld residuals.

As a secondary endpoint, lung cancer–specific survival was assessed using a competing-risk approach from the time of surgery to the time of lung cancer death.[26] Non–lung cancer deaths were considered a competing-risk event. Lung cancer cumulative incidence of death

(LC-CID) was compared between groups using Gray's test and quantified using Fine and Gray competing-risk regression. Relationships between ACT and LC-CID within each group were quantified as sub-distribution hazard ratios (SHRs) using competing-risk regression, stratified by p-stage. In the comparisons of interest between no ACT vs ACT for each time-to-event endpoint, ACT serves as the reference group, and we quantify the hazard of death without ACT compared to ACT with hazard ratios. A hazard ratio (HR) of less than 1 indicates that no ACT has a lower hazard of the event compared to ACT, while a HR greater than 1 indicates that no ACT has a greater hazard of the event compared to ACT (i.e., ACT is protective).

All analyses were two-sided; $P<0.05$ was considered statistically significant. Statistical analyses were conducted using Stata 15.0 (Stata Corp, College Station, TX) and R 3.6.1 (R Development Core Team, Vienna, Austria). Box and whisker plots were generated using a special version of Spotfire for quantitative pathology (TIBCO, Palo Alto, CA). Bar graphs were generated using GraphPad Prism version 9.0.2 (San Diego, CA).

RESULTS

PD-L1 Expression and Benefit from ACT

Patient demographic and clinical characteristics are listed in Table 1. Of 394 patients with stage II–III lung adenocarcinoma who underwent complete resection (R0), 211 (54%) received ACT, and 183 (46%) did not. Among patients who received ACT, 95% received platinum-based chemotherapy, 95% received ≥2 cycles of chemotherapy, and 81% completed all cycles of chemotherapy. Consistent with published data, ACT was associated with improved OS (hazard ratio [HR], 1.72; 95% CI, 1.35–2.21; $P<0.001$) (Figure 1A). Cell counts of lymphoid origin ($CD57^+$ natural killer cells, $CD20^+$ B cells, $CD4^+$ helper T cells, and $CD8^+$ cytotoxic T cells) and myeloid origin (MPO^+ neutrophils and $CD68^+$ and/or $CD163^+$ TAMs) in the TME were not statistically significantly different between patients who received ACT and those who did not (Figure 1B; Figure, Supplemental Data 3). Stratification based on PD-L1 expression on tumor cells only (cutoff value ≥1%, in accordance with current clinical practice; Table, Supplemental Data 1)[27] was not predictive for the overall cohort (data not shown), ACT cohort (HR, 1.79; 95% CI, 0.55–1.14; $P=0.44$), or no-ACT cohort (HR, 1.14; 95% CI, 0.81–1.60; $P=0.20$) (Figure 1C). High numbers of $PD\text{-}L1^+$ TAMs (at or above the median) were observed in 53% of patients with low (<1%) PD-L1 expression on tumor cells and 51% of patients with high (≥1%) PD-L1 expression on tumor cells (Figure 1D).

Patients were stratified into 4 groups based on PD-L1 expression on TAMs and tumor cells (Figure 1D; Table, Supplemental Data 2). Among patients with high PD-L1 expression on TAMs (Group 1; $P=0.049$) or tumor cells (Group 2; $P<0.001$), OS was statistically significantly better in those who received ACT. The results of Cox analysis, stratified by p-stage, confirmed this observation (no-ACT vs ACT, Group 1: HR, 1.79; 95% CI, 1.12–2.85; $P=0.015$; Group 2: HR, 3.02; 95% CI, 1.69–5.40; $P<0.001$). Patients with high PD-L1 expression on both Tumor cells and TAMs (Group 3) also had improved OS with ACT ($P=0.066$), although this association was not statistically significant. Results of analysis stratified by p-stage reflected this result (no-ACT vs ACT, Group 3: HR, 1.73; 95% CI,

1.0–2.98; *P*=0.051). Among patients with low/no PD-L1 expression on TAMs and tumor cells (Group 4), ACT was not associated with+ improved OS (no-ACT vs ACT, *P*=0.535; HR, 1.13; 95% CI, 0.69–1.84; *P*=0.627) (Figure 1E and F).

High MLR and Risk of Death without ACT

We next investigated the association between MLR (stratified by median, 0.39) and ACT status and OS benefit (Figure 2A and 2B). An MLR at or above the median (high myeloid content or low lymphoid content) was associated with a higher risk of death in the no-ACT cohort (HR, 1.62; 95% CI, 1.14–2.31; *P*=0.007) (Figure 2B) but not in the ACT cohort, which, in general, had better prognosis than the no-ACT cohort.

Patients with high PD-L1 expression on TAMs and high MLR had an OS benefit from ACT (Group 1: *P*=0.001; Group 3: *P*=0.021); this observation was confirmed in p-stage–adjusted Cox analysis (Group 1: HR, 3.87; 95% CI, 1.79–8.37; *P*<0.001; Group 3: HR, 2.19; 95% CI, 1.02–4.71; *P*=0.044). Patients with high PD-L1 expression on tumor cells and low MLR also had an OS benefit from ACT (*P*=0.002), which was also confirmed in p-stage–adjusted Cox analysis (Group 2: HR, 3.90; 95% CI, 1.59–9.52; *P*=0.003). Patients with low/no PD-L1 expression did not benefit from ACT, regardless of MLR status (Group 4, Figure 2C and 2D).

We next investigated the association between MLR and LC-CID. Similar to the relationships observed for OS, ACT was beneficial in Group 1 patients with high MLR (*P*=0.027), and this association was confirmed in p-stage–adjusted competing-risk analysis (SHR, 3.23; 95% CI, 1.35–7.73; *P*=0.009) (Figure 3A and 3B). Similar to the patterns observed for OS, patients with high PD-L1 expression on tumor cells and low MLR had a non-statistically significant benefit with ACT (SHR, 2.32; 95% CI, 0.88–6.15; *P*=0.091). Regardless of MLR status, patients with low/no PD-L1 expression did not benefit from ACT (Figure 3A and 3B).

Stromal Infiltration of MDSCs and Benefit from ACT

With the observation that high PD-L1 expression on TAMs and high MLR was associated with better OS and LC-CID, we assessed individual immune-cell counts in all cores (see Figure, Supplemental Data 4). Statistically significant differences among the four groups were observed in CD8 (Kruskal-Wallis *P*=0.010) and CD4 (*P*=0.036) cell counts. The results of Dunn's multiple comparison test revealed the differences in immune-cell counts were driven by individual groups, with no clear patterns across groups (see Figure, Supplemental Data 4A–C). CD68/163 counts in tumor (*P*=0.048) and stroma (*P*=0.005) differed across all groups. However, only in stroma were the differences between Groups 1 (*P*-adj=0.014) and 3 (*P*-adj=0.019) and Group 2 (see Figure, Supplemental Data 4C) statistically significant.

Given the above differences in stromal TAM counts between patients with high PD-L1 expression on TAMs (Groups 1 and 3), we attempted to identify the MDSC population that contributed to the higher MLR in the TME. We observed that a high stromal MDSC ratio, derived as the ratio of immature MDSCs ($CD11b^{+}CD33^{+}$ HLA-DR^{-}) to monocyte-like MDSCs ($CD33^{+}$ HLA-DR^{-}) at or above the median,[28–30] was associated with a benefit from ACT among patients with high PD-L1 expression on TAMs or tumor cells (Group

1: *P*=0.014; Group 2: *P*=0.003). A similar benefit was observed in Group 3, but this was not statistically significant (*P*=0.066) (Figure 4A). On p-stage–adjusted analyses, patients with high PD-L1 expression of any kind and high stromal MDSC ratio who did not receive ACT had a higher risk of death (Group 1: HR, 2.53; 95% CI, 1.29–4.96; *P*=0.007; Group 2: HR, 3.90; 95% CI, 1.57–9.68; *P*=0.003; Group 3: HR, 3.21; 95% CI, 1.23–8.35; *P*=0.017) (Figure 4A). Survival curves by tumor MDSC ratio are shown in Figure, Supplemental Data 5.

Similar to OS, patients with high PD-L1 expression on TAMs and high stromal MDSC ratio had a lower LC-CID with ACT (Group 1: *P*=0.025; Group 3: *P*=0.047) (Figure 4B). After adjustment for p-stage, patients with high PD-L1 expression on TAMs and high stromal MDSC ratio had a statistically significant lower LC-CID with ACT (Group 1: SHR, 2.89; 95% CI, 1.29–6.47; *P*=0.010; Group 3: SHR, 3.08; 95% CI, 1.13–8.42; *P*=0.028).

2-yr and 5-yr Survival

Patient stratification based on PD-L1 overexpression on TAMs and tumor cells distinguished patients with differences in 2-year OS of 55% vs 95% and 5-year OS of 20% vs 75% between those who did not and did receive ACT (Figure, Supplemental Data 6).

DISCUSSION

We previously established that effector and suppressor immune markers are independently prognostic in early-stage lung adenocarcinoma and squamous cell cancers.[9,31] The present study represents a comprehensive investigation of patients with locoregionally advanced stage II–III lung adenocarcinomas, with attention to immune markers in tumor and stromal compartments and their association with the clinical outcomes of OS, LC-CID, and stage-adjusted risk ratio. Our approach to investigate the resected tumor TME as a proxy for the premetastatic niche provides insights to help guide the development of combination chemoimmunotherapeutic strategies for patients with locoregionally advanced lung adenocarcinoma.

The limited survival benefit of 4%–15%[3,4] with ACT, currently recommended for all patients with stage II–II lung adenocarcinomas, is compounded by the side effects of ACT, which underscore the need for biomarkers of ACT response.[32,33] Unlike in advanced NSCLC, where investigation is limited by the availability of biopsy tissue, in stage II–III lung adenocarcinomas, fully resected specimens provide sufficient tissue to study the TME. Following the establishment of PD-L1 expression on tumor cells as a marker of therapy response in advanced NSCLC, it was investigated as a marker of chemotherapy response; the results, however, were inconclusive.[16,34] Recent preclinical and clinical investigations convincingly demonstrate that PD-L1 expression on immune cells, in addition to tumor cells, is necessary for response to ICI agents.[12,35] More importantly, PD-L1 expression on TAMs is predictive of response to ICI therapy in NSCLC, melanoma, ovarian cancer, and triple-negative breast cancer.[36–39] Our study demonstrates that patient stratification based on PD-L1 overexpression on TAMs and tumor cells can distinguish patients with a 2-year OS of 95% vs 55% and 5-year OS of 75% vs 20% between those who did and did not receive ACT. Furthermore, our observation that high PD-L1 expression on TAMs alone

can be a marker for selection and stratification is intriguing and underscores the need for assessment of PD-L1 expression on TAMs, in addition to tumor cells. To further illustrate the translatability of our findings, we have demonstrated that PD-L1 expression on TAMs can be assessed using the reflex immunohistochemistry testing currently performed for assessment of PD-L1 expression on tumor cells (Figure, Supplemental Data 7). Therefore, use of PD-L1 expression on TAMs and tumor cells as markers of response to ACT is immediately and highly feasible.

The LACE-Bio study investigators analyzed PD-L1 expression on tumor cells and immune cells in patients with early-stage NSCLC and, contrary to our observations, concluded that PD-L1 expression is not predictive.[16] However, there are several differences between the studies. Although both studies are retrospective in nature and use the same antibody for PD-L1 expression, in the LACE-Bio study, one-third of patients had stage I disease (none in our study), only 41% of patients had lung adenocarcinomas (100% in our study), patients were predominantly men (73% vs 35% in our study), 31% of patients underwent pneumonectomy (8.4% in our study), and 51% of patients had N0 status (20% in our study).[16] Whereas the LACE-Bio study investigated PD-L1 expression on all immune cells, we assessed PD-L1 expression on TAMs only on the basis of the strong rationale provided by recently published data.[11–13,16] These differences, combined with our published results that highlight the differential immune composition between lung adenocarcinoma and squamous cell cancer, underscore the need for assessment of immune markers by histologic subtype rather than by all NSCLCs combined.[9,31] The high-throughput evaluation of multiple markers in both tumor and tumor-associated stroma, a key requisite for assessment of immune responses, is a strength of our study. Emerging data by investigation of human non-small cell lung cancer cells and peripheral blood monocytes demonstrate differential PD-L1 expression on immune cells (macrophages, dendritic cells, and MDSCs) influenced by specific cytokines such as IL-1a, IL-10, IL-27, and IL-32g in addition to IFN-γ, which can explain the differences observed in PD-L1 expression on TAMs alone in our study in contrast to PD-L1 on all immune cells in LACE-Bio study. In addition, the variable results among both studies could have originated from the study cohort—a homogenous cohort of adenocarcinoma in our study versus heterogenous non-small cell lung cancer in LACE-BIO study.[40]

As high PD-L1 expression on TAMs and high myeloid content were associated with benefit from ACT, and as these patients also had high stromal TAM counts, we suspected the involvement of factors such as MDSCs, which are immature myeloid precursors that are systemically recruited and subsequently converted to immunosuppressive TAMs after entry into the TME.[28,41,42] In our analysis of the tumor and stromal compartments independently, we observed that the ratio of immature MDSCs to monocyte-like MDSCs (surrogate for MDSC infiltration) in tumoral stroma was similarly predictive as MLR, suggesting that peripherally circulating immunosuppressive TAM precursors can perhaps be mitigated by the administration of ACT.[18,20,43] Importantly, the increased myeloid content appeared to be associated with PD-L1 expression on TAMs, which is indicative of IFN-γ release and immune activation, whereas PD-L1 expression on tumor cells corresponds with epigenetic dysregulation of the PD-L1 gene, tumor promoter demethylation, or gene amplification, in addition to inflammatory factors.[13,36,37] With consideration of these observations combined with recent publications of chemotherapy immune modulation in patients with thoracic

cancers,[44,45] we postulate that the myelosuppressive effects of chemotherapy on MDSCs and other protumor immune cells may contribute to the survival benefit from ACT. Our observations are further strengthened by recent evidence that tissue-resident macrophages provide a pre-metastatic niche to early NSCLC cells.[46,47]

The limitations of our study include its retrospective nature and lack of an internal validation set with which to construct a predictive model. In addition, only 81% of patients who received ACT completed all four cycles of chemotherapy, as was commonly seen in ACT trials.[48] Although it is thought that four cycles of ACT are required to achieve cytotoxic effects, the necessary number of cycles to achieve immunomodulatory effects is unknown.[49,50] In addition, the cut-off values used for TAMs and MLR (median) in this retrospective study are exploratory, not confirmatory. Future studies will set the cut-off value using ROC curves. A prospective study designed to validate our results, along with analyses of matched peripheral blood samples to assess associations of stromal MDSC ratio and MLR with circulating MDSCs and neutrophil-lymphocyte ratio, respectively, is planned. Such a study can identify the number of cycles of ACT that are required to achieve immunomodulatory effects and can inform the development of rational regimens of combination chemoimmunotherapy. The significance of our study is even more important in induction and/or adjuvant chemoimmunotherapy wherein the chemotherapy may enhance the ICI agent efficacy by modulating the tumor immune microenvironment. Examination of the full histological slide is necessary in those studies due to expected necrosis, and to complete better pathological responses.

In conclusion, PD-L1 expression on TAMs and tumor cells is associated with benefit from ACT—in particular, in patients with high MLR or stromal MDSC infiltration in the TME. This association suggests that analysis of the resected TME can inform postresection treatment. Importantly, ACT does not appear to benefit patients without substantial PD-L1 expression on TAMs or tumor cells, regardless of MLR or MDSC ratio. This suggests that the tumor cytotoxicity and immunomodulation of ACT are not associated with improved survival in patients without immune activation. These observations from our retrospective study are to be validated in external cohorts as well as in prospective studies evaluating immune cell function in addition to immune cell marker expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest Statement:

Dr. Adusumilli declares research funding from ATARA Biotherapeutics; Scientific Advisory Board Member and Consultant for ATARA Biotherapeutics, Bayer, Carisma Therapeutics, Imugene, ImmPactBio, Takeda Therapeutics; Patents, royalties and intellectual property on mesothelin-targeted CAR and other T-cell therapies licensed to ATARA Biotherapeutics, issued patent method for detection of cancer cells using virus, and pending patent applications on PD-1 dominant negative receptor, wireless pulse-oximetry device, and on an ex vivo malignant pleural effusion culture system. All other authors do not have conflicts of interest to disclose.

Memorial Sloan Kettering Cancer Center (MSK) has licensed intellectual property related to mesothelin-targeted CARs and T-cell therapies to ATARA Biotherapeutics, and has associated financial interests.

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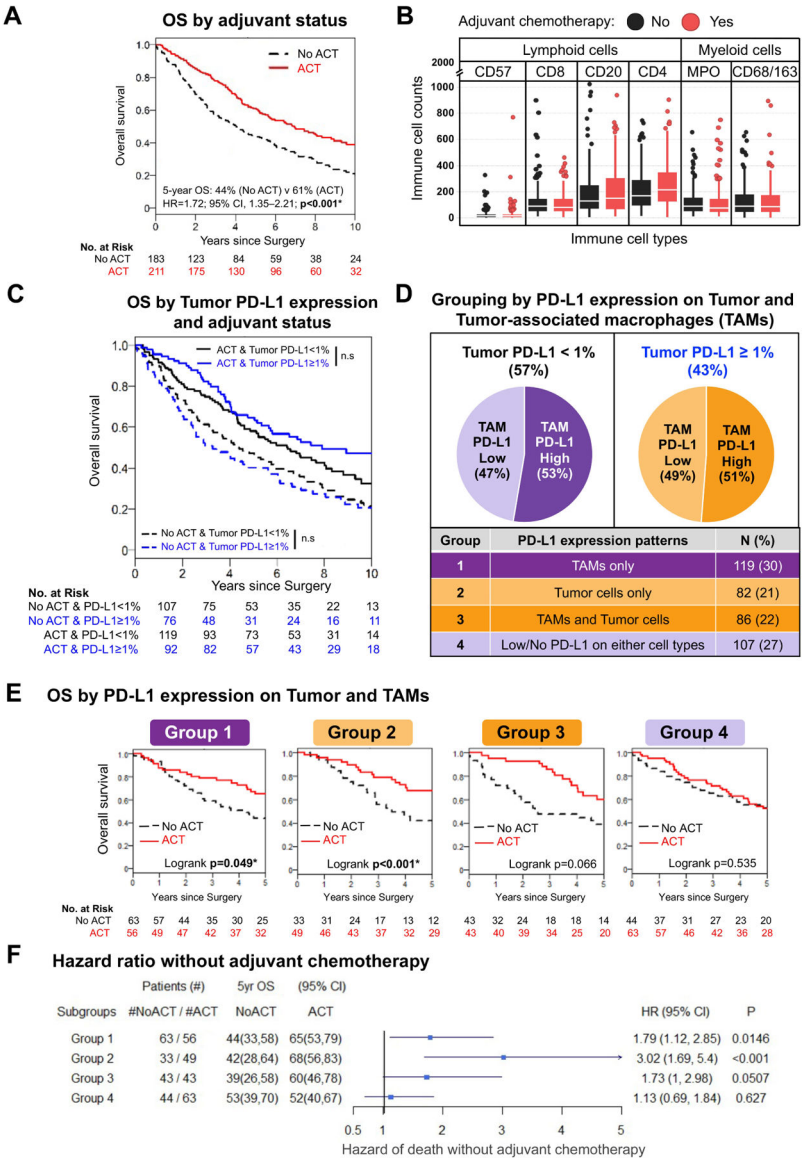

Figure 1.

Survival benefit from adjuvant chemotherapy (ACT) by programmed death ligand-1 (PD-L1) expression on tumor-associated macrophages (TAMs) and tumor cells (Tumor cells). (A) Kaplan-Meier curves of patients stratified by ACT status demonstrating a statistically significant improvement in overall survival (OS) among patients who received ACT (5-year OS: no ACT vs ACT, 44% vs 61%; $P<0.001$). (B) Distribution of immune cell counts of lymphoid (CD57, CD8, CD20, CD4) or myeloid (MPO, CD68/CD163) cells, stratified by ACT (red) or no ACT (black). Each boxplot displays the median (horizontal line within the box) and interquartile range (height of box). (C) Kaplan-Meier curves of patients stratified by PD-L1 expression on Tumor cells only (cutoff ≥1%) showing no association with OS benefit in both ACT and no-ACT cohorts. (D) Pie charts and table displaying the number and percentage of patients within each of the four groups based on PD-L1 expression on Tumor cells and TAMs. (E) Kaplan-Meier curves demonstrating the association between

OS and ACT in Group 1 (high PD-L1 expression on TAMs; P=0.049) and Group 2 (high PD-L1 expression on Tumor cells; P<0.001). A nonstatistically significant benefit from ACT was observed in Group 3 (high PD-L1 on TAMs and Tumor cells; P=0.066), and ACT was not associated with a survival benefit in Group 4 (low/no PD-L1 expression on TAMs or Tumor cells; P=0.535). (F) Forest plot summarizing results from pathologic stage–adjusted analyses, demonstrating an increased hazard of death among patients who did not receive ACT in Group 1 (hazard ratio [HR], 1.79; 95% CI, 1.12–2.85; P=0.015), Group 2 (HR, 3.02; 95% CI, 1.69–5.40; P<0.001), and Group 3 (HR, 1.73; 95% CI, 1.00–2.98; P=0.051). The relationship between ACT and hazard of death was not statistically significant for Group 4 (HR, 1.13; 95% CI, 0.69–1.84; P=0.627). *Statistically significant (P<0.05); n.s., not significant.

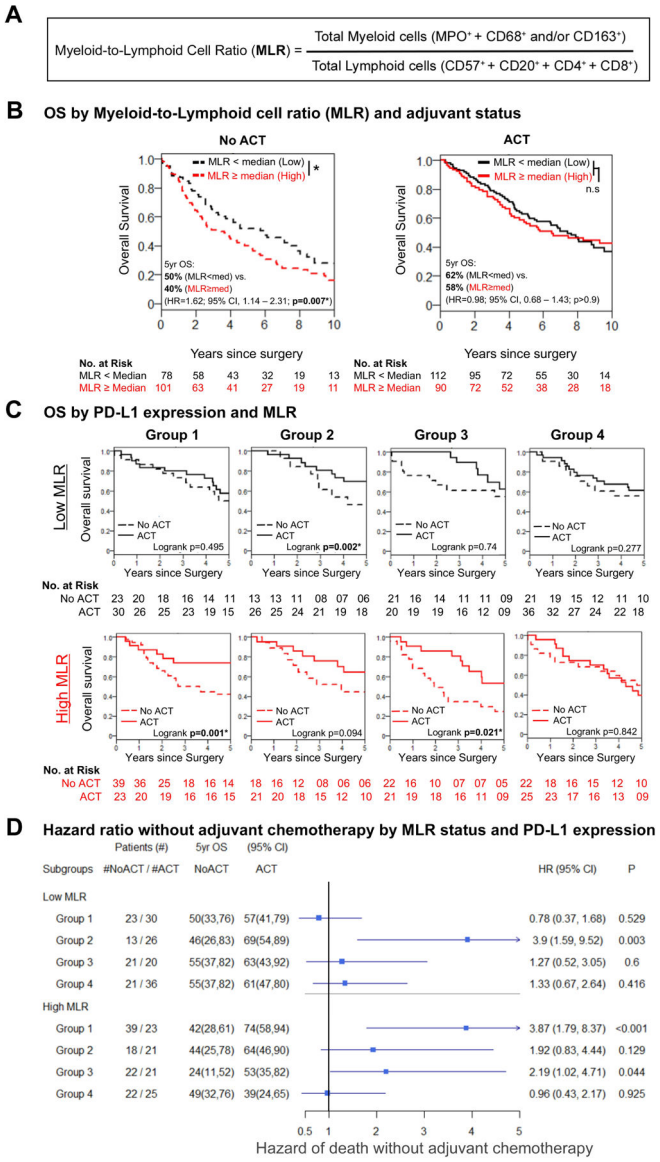

Figure 2.

Association between survival benefit from adjuvant chemotherapy (ACT) and high programmed death ligand-1 (PD-L1) expression and high myeloid-lymphoid ratio (MLR). (A) MLR is derived from the ratio of total myeloid cells to lymphoid cells. Total myeloid cells are equal to the sum of neutrophils (MPO^+ cells) and tumor-associated macrophages ($CD68^+$ and/or $CD163^+$ cells). Total lymphoid cells are equal to the sum of natural killer, B, helper T, and cytotoxic T cells (identified by $CD57^+$, $CD20^+$, $CD4^+$, and $CD8^+$ markers, respectively). (B) Kaplan-Meier survival estimate curves demonstrating a statistically significant association between MLR and overall survival (OS). In the no-ACT cohort, survival of patients with high MLR was statistically significantly worse (5-year OS: MLR low vs. high, 50% vs. 40%; P=0.007). Whereas survival was not significantly different in the ACT cohort by MLR status (5-year OS: MLR low vs. high, 62% vs. 58%; P>0.9), patients with high MLR benefitted more from ACT, compared with those with low MLR

(5-year OS: high MLR, no ACT vs. ACT, 40% vs. 58%; low MLR, no ACT vs ACT, 50% vs. 62%). (C) Kaplan-Meier survival estimates stratified by PD-L1 expression and MLR showing an improvement in survival with ACT among patients with high PD-L1 expression on tumor-associated macrophages and high MLR (Groups 1 and 3; Group 1/high MLR, log-rank P=0.001; Group 3/high MLR, log-rank P=0.021). Patients with high PD-L1 expression on tumor cells and low MLR benefited from ACT (Group 2/low MLR, log-rank P=0.002). Patients with low/no PD-L1 expression had no improvement in OS with ACT. (D) Pathologic stage–adjusted hazard ratio (HR) plot showing an increased risk of death without ACT only among patients in Group 2 with low MLR (Group 2/low MLR, HR, 3.9; 95% CI, 1.59–9.52; P=0.003) and patients in Groups 1 and 3 with high MLR (Group 1/high MLR, HR, 3.87; 95% CI, 1.79–8.37; P<0.001; Group 3/high MLR, HR, 2.19; 95% CI, 1.02–4.71; P=0.044). Regardless of MLR status, patients in Group 4 did not have an increased risk of death without ACT. *Statistically significant (P<0.05); n.s., not significant.

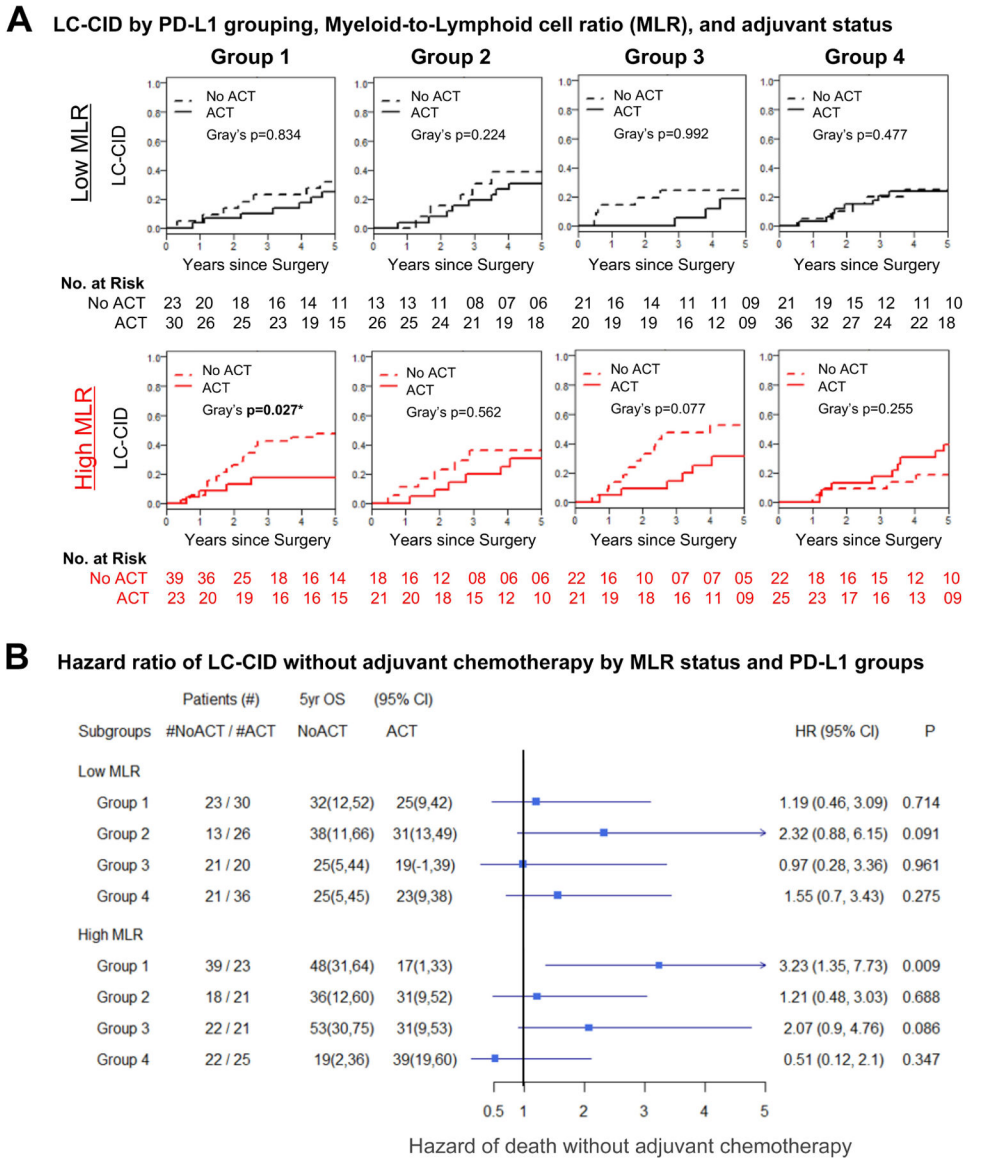

Figure 3.

Adjuvant chemotherapy (ACT) and lung cancer cumulative incidence of death (LC-CID). (A) Kaplan-Meier curves showing significant improvement in LC-CID among patients with high programmed death ligand-1 (PD-L1) expression on tumor-associated macrophages and high myeloid-lymphoid ratio (MLR) who received ACT (Group 1/high MLR, Gray's *P*=0.027). A delayed trend of LC-CID, in general, can be seen in all groups of patients who received ACT, except for patients in Group 4, regardless of MLR status. (B) Forest plot of pathologic stage–adjusted subhazard ratios (SHRs) showing a significant hazard of lung cancer–related death associated with not receiving ACT among patients in Group 1 with high MLR (Group 1/high MLR, SHR, 3.23; 95% CI, 1.35–7.73; *P*=0.009). A nonstatistically significant improvement in LC-CID was seen in Group 3 patients with high MLR (Group 3/high MLR, SHR, 2.07; 95% CI, 0.90–4.76; *P*=0.086) and in Group 2 patients with low

MLR (Group 2/low MLR, SHR, 2.32; 95% CI, 0.88–6.15; P=0.091). HR, hazard ratio; OS, overall survival. *Statistically significant (P<0.05).

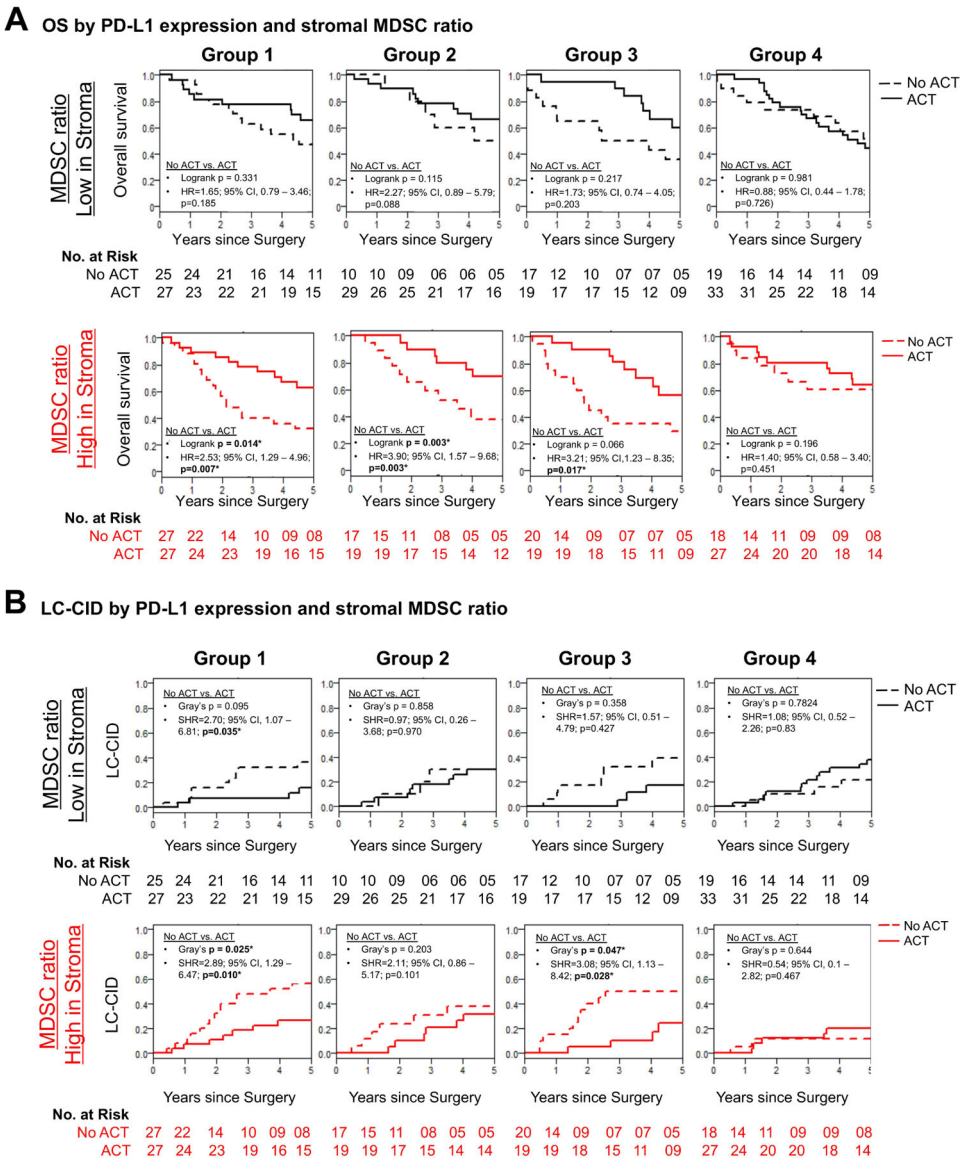

Figure 4.

Association between survival benefit from adjuvant chemotherapy (ACT) and high stromal myeloid-derived suppressor cell (MDSC) ratio in patients with high programmed death ligand-1 (PD-L1) expression on tumor-associated macrophages (TAMs) and tumor cells (Tumor cells). (A) Kaplan-Meier survival estimate of patients across PD-L1 groups stratified by low and high stromal MDSC ratio. Patients with high stromal MDSC ratio and high PD-L1 expression on TAMs or Tumor cells benefited from ACT (Group 1/high stromal MDSC ratio, log-rank *P*=0.014; Group 2/high stromal MDSC ratio, log-rank *P*=0.03). A similar trend was observed in patients with PD-L1 expression on both Tumor cells and TAMs, irrespective of stromal MDSC ratio. Pathologic stage–adjusted hazard ratio (HR) plot showing an increased risk of death associated with the absence of ACT among patients with high PD-L1 expression on TAMs and/or Tumor cells and high MDSC ratio in Groups 1, 2, and 3 (Group 1/high stromal MDSC ratio, log-rank *P*=0.007; Group 2/high stromal

MDSC ratio, log-rank P=0.003; Group 3/high stromal MDSC ratio, log-rank P=0.017). For patients in Group 4, regardless of stromal MDSC ratio, absence of ACT was not associated with a statistically significantly higher risk of death. (B) Kaplan-Meier survival plots of patients with high PD-L1 expression on TAMs and high stromal MDSC ratio demonstrating a statistically significant benefit from ACT (Group 1/high stromal MDSC ratio, Gray's P=0.025; Group 3/high stromal MDSC ratio, Gray's P=0.047). Pathologic stage–adjusted Cox proportional subhazard ratio analyses demonstrate an increased risk of lung cancer cumulative incidence of death (LC-CID) without ACT in patients with high PD-L1 expression on TAMs and high stromal MDSC ratio (Group 1/high stromal MDSC ratio, Gray's P=0.01; Group 3/high stromal MDSC ratio, Gray's P=0.028). No increased risk of lung cancer–related death was observed in patients with high PD-L1 expression on TAMs or Tumor cells and low stromal MDSC ratio. In contrast, in patients with low/no PD-L1 expression, lack of ACT was not associated with an increased risk of lung cancer–related death, regardless of stromal MDSC ratio. OS, overall survival. *Statistically significant (P<0.05).

Table 1.

Demographics and clinical characteristics

Characteristic	Group 1 (TAM PD-L1) (N=119; 30%)	Group 2 (tumor PD-L1) (N=82; 21%)	Group 3 (TAM & tumor PD-L1) (N=86; 22%)	Group 4 (No PD-L1) (N=107; 27%)	*P*
Age, years	68.9 (60.4–75.1)	67.3 (63.0–74.0)	66.9 (59.4–74.6)	70.0 (63.1–75.9)	0.3
Sex					
Female	77 (65)	57 (70)	50 (58)	76 (71)	0.3
Male	42 (35)	25 (30)	36 (42)	31 (29)	
Smoking status					
Never	25 (21)	9 (11)	9 (10)	30 (28)	**0.013**
Former	75 (63)	60 (73)	67 (78)	67 (63)	
Current	19 (16)	13 (16)	10 (12)	10 (9.3)	
Pack-years	30.0 (5.0–53.8)	39.5 (17.0–54.0)	34.5 (20.0–57.5)	26.0 (0.0–52.5)	0.13
COPD (N=393)					
No	102 (86)	62 (76)	66 (78)	90 (84)	0.2
Yes	17 (14)	20 (24)	19 (22)	17 (16)	
Resection					
Pneumonectomy	10 (8.4)	5 (6.1)	5 (5.8)	6 (5.6)	1
Bilobectomy	4 (3.4)	4 (4.9)	3 (3.5)	4 (3.7)	
Lobectomy	98 (82)	66 (80)	73 (85)	89 (83)	
Segmentectomy	7 (5.9)	7 (8.5)	5 (5.8)	8 (7.5)	
FEV1 (N=389)	85.0 (74.0–99.0)	80.0 (70.0–97.0)	81.0 (69.9–99.5)	87.5 (73.0–100.0)	0.2
DLCO (N=376)	84.0 (68.5–97.0)	70.0 (63.5–83.2)	76.5 (60.0–92.0)	80.5 (67.0–90.5)	**0.008**
Tumor SUV max (N=334)	7.2 (4.0–9.5)	6.1 (4.0–9.8)	7.3 (3.5–10.5)	6.3 (3.1–10.1)	0.8
Tumor size, cm	2.8 (2.2–5.1)	2.8 (1.8–4.5)	3.2 (2.0–4.5)	3.0 (2.1–4.5)	0.7
Invasive tumor size, cm	2.7 (2.0–4.5)	2.8 (1.8–4.5)	3.1 (2.0–4.1)	2.7 (1.9–4.3)	0.8
Nodal status					
Negative	24 (20)	10 (12)	10 (12)	16 (15)	0.3
Positive	95 (80)	72 (88)	76 (88)	91 (85)	
T stage					
1	38 (32)	32 (39)	29 (34)	36 (34)	0.8
2	41 (34)	28 (34)	37 (43)	40 (37)	
3	30 (25)	18 (22)	13 (15)	23 (21)	
4	10 (8.4)	4 (4.9)	7 (8.1)	8 (7.5)	
p-stage (8^{th} edition)					
IIB	62 (52)	47 (57)	39 (45)	48 (45)	0.5
IIIA	51 (43)	28 (34)	40 (47)	52 (49)	
IIIB	6 (5.0)	7 (8.5)	7 (8.1)	7 (6.5)	
LVI (N=393)					
Absent	37 (31)	24 (29)	24 (28)	26 (25)	0.7
Present	82 (69)	58 (71)	62 (72)	80 (75)	
Necrosis (N=381)					

Characteristic	Group 1 (TAM PD-L1) (N=119; 30%)	Group 2 (tumor PD-L1) (N=82; 21%)	Group 3 (TAM & tumor PD-L1) (N=86; 22%)	Group 4 (No PD-L1) (N=107; 27%)	*P*
No	79 (68)	57 (73)	49 (59)	85 (82)	**0.006**
Yes	37 (32)	21 (27)	34 (41)	19 (18)	
Mutation type (N=241)					
Wild type	29 (49)	28 (46)	30 (64)	37 (50)	0.069
EGFR	10 (17)	6 (10)	4 (8.5)	18 (24)	
KRAS	20 (34)	27 (44)	13 (28)	19 (26)	
STAS (N=385)					
No	44 (38)	27 (34)	29 (34)	37 (36)	0.9
Yes	72 (62)	52 (66)	57 (66)	67 (64)	
Histologic subtype					
Lepidic	5 (4.2)	2 (2.4)	1 (1.2)	1 (0.9)	**0.015**
Acinar	46 (39)	23 (28)	31 (36)	52 (49)	
Papillary	17 (14)	10 (12)	8 (9.3)	13 (12)	
Micropapillary	17 (14)	8 (10)	7 (8.1)	17 (16)	
Solid	30 (25)	37 (45)	37 (43)	19 (18)	
Invasive mucinous	3 (2.5)	2 (2.4)	1 (1.2)	5 (4.7)	
Colloid	1 (0.8)	0 (0)	1 (1.2)	0 (0)	
Adjuvant therapy					
No	63 (53)	33 (40)	43 (50)	44 (41)	0.2
Yes	56 (47)	49 (60)	43 (50)	63 (59)	
Immune cell counts					
CD57	13 (0–773)	12 (2–125)	16 (0–194)	11 (0–328)	0.092
CD8	86 (10–806)	84 (12–460)	103 (10–900)	70 (7–478)	**0.010**
CD20	115 (3–1249)	159 (9–2076)	168 (9–1025)	129 (6–1449)	0.28
CD4	184 (13–2417)	238 (16–1217)	190 (5–725)	201 (15–1106)	**0.036**
MPO	85 (9–754)	75 (9–694)	82 (8–461)	80 (11–596)	0.97
CD68/163	98 (6–627)	84 (8–1211)	104 (7–862)	78 (0–495)	0.080
MLR	0.5 (0.2–0.9)	0.4 (0.2–0.8)	0.4 (0.2–0.7)	0.3 (0.2–0.6)	0.4
Median MLR					
Below	57 (48)	43 (52)	43 (50)	63 (59)	0.4
Above	62 (52)	39 (48)	43 (50)	44 (41)	

NOTE. Data are median (interquartile range) or no. (%).

Abbreviations: COL, colloid carcinoma; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 second; IMA, invasive mucinous adenocarcinoma; LVI, lymphovascular invasion; MLR, myeloid-lymphoid ratio; p-stage, pathologic stage; STAS, spread through air spaces; SUVmax, maximum standardized uptake value. The four groups based on PD-L1 expression on Tumor cells and Tumor-associated macrophages (TAMs) are Group 1 (high PD-L1 expression on TAMs), Group 2 (high PD-L1 expression on Tumor cells), Group 3 (high PD-L1 on TAMs and Tumor cells), and Group 4 (low/no PD-L1 expression on TAMs or Tumor cells).