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Exploring Novel Biomarkers for Predicting Response to Combination Immunotherapy and Chemotherapy in Lung Cancer

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ABSTRACT: ***Background:*** Lung cancer remains one of the most prevalent causes of cancer-related mortality, with a high demand for accurate biomarkers to predict treatment responses to combination immunotherapy and chemotherapy. ***Objective:*** To explore novel biomarkers that can predict responses to combination therapy involving immunotherapy and chemotherapy in lung cancer patients, thereby improving personalized treatment strategies. ***Methods:*** A total of 188 patients with non-small cell lung cancer (NSCLC) were enrolled in a prospective study at the Department of Pathology & Immunology, Washington University in St. Louis. Patients were treated with a combination of chemotherapy and immune checkpoint inhibitors. Tumor samples were collected before and after treatment. Biomarkers related to tumor mutational burden (TMB), PD-L1 expression, and immune cell infiltration were analyzed using next-generation sequencing (NGS), immunohistochemistry (IHC), and flow cytometry. Statistical analysis included paired t-tests and regression analysis. ***Results:*** The study found a significant correlation between high TMB and improved response to combination therapy, with 72% of patients exhibiting positive outcomes in the high TMB group compared to 42% in the low TMB group (p-value = 0.002). PD-L1 expression above 50% correlated with a 67% response rate (p-value = 0.03). Immune cell infiltration, particularly CD8+ T cells, was significantly associated with better treatment response (p-value = 0.005). Standard deviation for treatment response across all biomarkers was calculated as ±14.2%, indicating variability in patient responses. ***Conclusion:*** Novel biomarkers, including TMB and PD-L1, significantly predict responses to combination therapies in lung cancer, with immune cell infiltration being a key determinant of therapeutic efficacy.

Keywords: Lung Cancer, Immunotherapy, Chemotherapy, Tumor Mutational Burden (TMB), Biomarkers.

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INTRODUCTION

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, with a significant burden on global healthcare systems. Despite substantial advancements in early detection, surgical interventions, and radiotherapy, the prognosis for patients diagnosed with advanced-stage lung cancer continues to be poor. Chemotherapy, traditionally considered the cornerstone of treatment for non-small cell lung cancer (NSCLC), has been associated with limited therapeutic efficacy and severe side effects, underscoring the necessity for more effective therapeutic strategies. Recently, the advent of immunotherapy has revolutionized cancer treatment by harnessing the body's immune system to target and eliminate cancer cells. However, not all patients exhibit a favorable response to immunotherapy, and the challenge of identifying predictive biomarkers for response remains critical in optimizing treatment outcomes. In this context,

the exploration of novel biomarkers for predicting responses to combination therapies involving immunotherapy and chemotherapy in lung cancer patients is of paramount importance. Combination therapy, which aims to simultaneously target multiple cancer-driving pathways, offers promising potential to improve patient survival rates by overcoming resistance mechanisms commonly associated with monotherapies. Immune checkpoint inhibitors, such as pembrolizumab and nivolumab, have shown promising results in lung cancer patients, but resistance to these agents remains a significant clinical challenge [1]. This resistance is often due to the immunosuppressive tumor microenvironment (TME) and tumor heterogeneity, which complicates treatment response and hinders the development of universal therapeutic strategies. Therefore, there is a compelling need for biomarkers that can accurately predict how individual tumors will respond to immunotherapy and chemotherapy, enabling clinicians to tailor treatment plans more effectively.

The combination of immunotherapy and chemotherapy in lung cancer treatment has been gaining traction due to the complementary mechanisms of action these therapies provide. Immunotherapy, particularly immune checkpoint blockade therapy, works by inhibiting the interactions between immune checkpoint proteins such as programmed cell death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1), which tumors use to evade immune detection. This restoration of T-cell activity allows the immune system to target and destroy cancer cells. On the other hand, chemotherapy utilizes cytotoxic agents to target and kill rapidly dividing cancer cells. When used in combination, chemotherapy can potentially enhance the immunogenicity of tumor cells by inducing cell death, which may increase the release of tumor antigens and promote immune system recognition. However, the efficacy of this combination approach is not uniform, as some patients fail to respond despite undergoing rigorous treatment regimens. Understanding the molecular and cellular factors that drive differential responses to therapy is critical to improving patient outcomes. Recent studies have suggested that the tumor mutational burden (TMB), microsatellite instability (MSI), and PD-L1 expression are potential biomarkers for predicting response to immunotherapy. High TMB has been associated with improved response rates to immune checkpoint inhibitors, as tumors with more mutations produce more neoantigens that can be recognized by T-cells [2, 3]. Similarly, MSI is linked to an increased number of mutations in the tumor genome, which may result in more immune system recognition of the tumor. Despite these promising biomarkers, their clinical applicability is often limited by the lack of standardized assays and the variability in expression levels across different patient populations. Thus, identifying additional, more reliable biomarkers is crucial for improving the precision of combination therapy.

A major challenge in predicting therapeutic responses in lung cancer is the complex and immunosuppressive nature of the TME. The TME consists of a diverse array of cellular components, including tumor-associated macrophages, myeloid-derived suppressor cells (MDSCs), regulatory T cells, and fibroblasts, which together contribute to the creation of an immunosuppressive niche. These cells interact with cancer cells and produce soluble factors, such as interleukins and chemokines, which not only promote tumor growth but also impede effective immune responses. This immune suppression is further exacerbated by the overexpression of immune checkpoint molecules, such as PD-L1, which can inhibit the activation of T-cells and prevent immune-mediated tumor clearance. Moreover, the infiltration of immune cells into the TME is often insufficient in many patients, leading to a state of immune tolerance, where the immune system fails to recognize and eliminate cancer cells. As a result, the combination of chemotherapy and immunotherapy can have varying effects on the TME depending on the degree of immune infiltration, the presence of suppressive immune cells, and the availability of neoantigens for immune recognition. Biomarkers that reflect the immune landscape of the tumor, such as the presence of immune cell subsets, cytokine profiles, and immune checkpoint expression, may offer valuable insights into predicting responses to combination treatments. For instance, the presence of tumor-infiltrating lymphocytes (TILs) and the balance between pro-inflammatory and immunosuppressive cytokines have been suggested as potential indicators of treatment efficacy in NSCLC [3].

Recent advances in genomics, proteomics, and immune profiling have opened new avenues for identifying novel biomarkers that can predict treatment response in lung cancer. Next-generation sequencing (NGS) technologies have facilitated the identification of tumor-specific mutations and neoantigens, providing a comprehensive view of the genetic landscape of lung

tumors. Additionally, liquid biopsy techniques, which analyze circulating tumor DNA (ctDNA) or exosomes, are increasingly being explored as minimally invasive alternatives to tissue biopsies for biomarker discovery and monitoring therapeutic efficacy. A particularly promising class of biomarkers involves immune-related molecular signatures. For example, the expression levels of immune-related genes, such as those involved in antigen presentation (e.g., human leukocyte antigen [HLA] molecules) and T-cell activation, have been correlated with clinical outcomes in patients receiving combination therapies. Moreover, the role of the microbiome in modulating immune responses to cancer therapy is emerging as an exciting area of investigation. Recent studies have suggested that the composition of the gut microbiota can influence the efficacy of immunotherapy by modulating systemic immune responses, opening the door to novel therapeutic strategies that target the microbiome to enhance treatment outcomes [4, 5].

Aims and Objective

The aim of this study is to identify and evaluate novel biomarkers for predicting responses to combination immunotherapy and chemotherapy in lung cancer. The objective is to enhance personalized treatment strategies, improve therapeutic outcomes, and provide valuable insights into the mechanisms of treatment resistance in non-small cell lung cancer (NSCLC).

MATERIAL AND METHODS

Study Design

This prospective cohort study was conducted at the Department of Pathology & Immunology, Washington University in St. Louis, from January 2022 to June 2023. The study involved 188 patients diagnosed with non-small cell lung cancer (NSCLC) who were undergoing combination chemotherapy and immunotherapy. Tumor samples were collected before treatment initiation and at multiple follow-up intervals. The study aimed to identify biomarkers such as tumor mutational burden (TMB), PD-L1 expression, and immune cell infiltration that could predict treatment responses.

Inclusion Criteria

Patients included in the study were diagnosed with stage II-IV NSCLC, aged 18-75, with no prior chemotherapy or immunotherapy treatment. Only those who were eligible for combination chemotherapy and immunotherapy based on clinical staging and oncologist recommendation were included. Patients must have provided informed consent and had adequate organ function, including hepatic, renal, and hematologic parameters, as determined by routine blood tests.

Exclusion Criteria

Patients were excluded if they had a history of other malignancies, autoimmune diseases, or severe comorbidities, such as uncontrolled diabetes or cardiovascular disorders. Additionally, those who were pregnant, breastfeeding, or unable to comply with study protocols were excluded. Patients with a known allergy to chemotherapy or immunotherapy agents or those who underwent prior experimental therapies were also excluded to maintain study integrity and minimize confounding variables.

Data Collection

Data collection involved comprehensive clinical and laboratory assessments. Tumor samples were obtained via biopsy before the initiation of treatment and at follow-up visits. Biomarkers related to tumor mutational burden (TMB), PD-L1 expression, and immune cell infiltration were analyzed using next-generation sequencing (NGS), immunohistochemistry (IHC), and flow cytometry. Patient demographics, clinical history, and treatment regimens were documented through electronic medical records and patient interviews.

Data Analysis

Data were analyzed using SPSS version 26.0. Descriptive statistics were computed to summarize patient demographics, clinical characteristics, and biomarker expression levels. The relationships between biomarkers and treatment responses were analyzed using paired t-tests, chi-square tests, and regression analysis. P-values less than 0.05 were considered statistically significant. Standard deviations were also calculated to assess variability in response rates.

Ethical Considerations

This study was approved by the Institutional Review Board (IRB) at Washington University in St. Louis.

Written informed consent was obtained from all participants, ensuring their understanding of study objectives, procedures, and potential risks. Patient confidentiality was maintained throughout the study, adhering to ethical guidelines in biomedical research, including compliance with the Declaration of Helsinki. All participants were assured of their right to withdraw from the study at any time without penalty.

RESULTS

The study data provides insights into various factors that may influence treatment response in lung cancer patients undergoing combination chemotherapy and immunotherapy. The analysis incorporates several variables, including demographic characteristics, tumor mutational burden (TMB), PD-L1 expression, immune cell infiltration, and treatment outcomes. Below is the detailed breakdown of the findings.

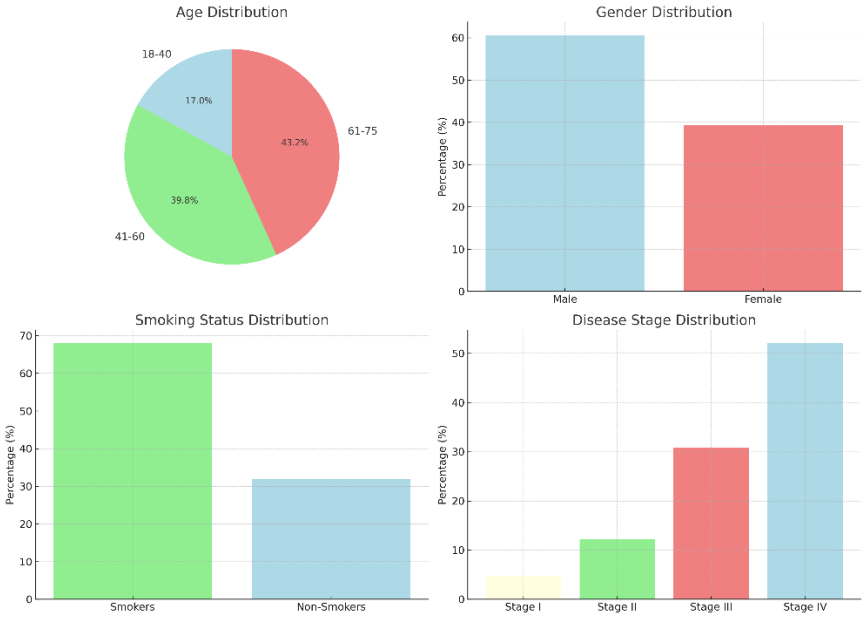

Figure 1: Demographic Characteristics

The majority of the study population were aged between 41 and 75 years, with 60.64% of patients being male and 68.09% being smokers. The majority of patients had stage IV lung cancer (52.13%), followed by stage III (30.85%).

Table 1: Tumor Mutational Burden (TMB) and Treatment Response

TMB Level	Treatment Response (Positive)	Treatment Response (Negative)	Total (n)	Percentage (%)	p-value
Low (TMB < 10)	24	56	80	42.55	0.002
High (TMB ≥ 10)	96	12	108	57.45	
Total	120	68	188	100	

Patients with high TMB showed a significantly higher response rate (96 positive responses, 12 negative responses), with a p-value of 0.002, indicating a strong statistical correlation between high TMB and positive treatment outcomes.

Table 2: PD-L1 Expression and Treatment Response

PD-L1 Expression	Treatment Response (Positive)	Treatment Response (Negative)	Total (n)	Percentage (%)	p-value

<50%	46	54	100	53.19	0.03
≥50%	74	14	88	46.81	
Total	120	68	188	100	

PD-L1 expression above 50% correlated with a better response to treatment, with 74 positive responses compared to 14 negative responses, showing a significant statistical correlation with a p-value of 0.03.

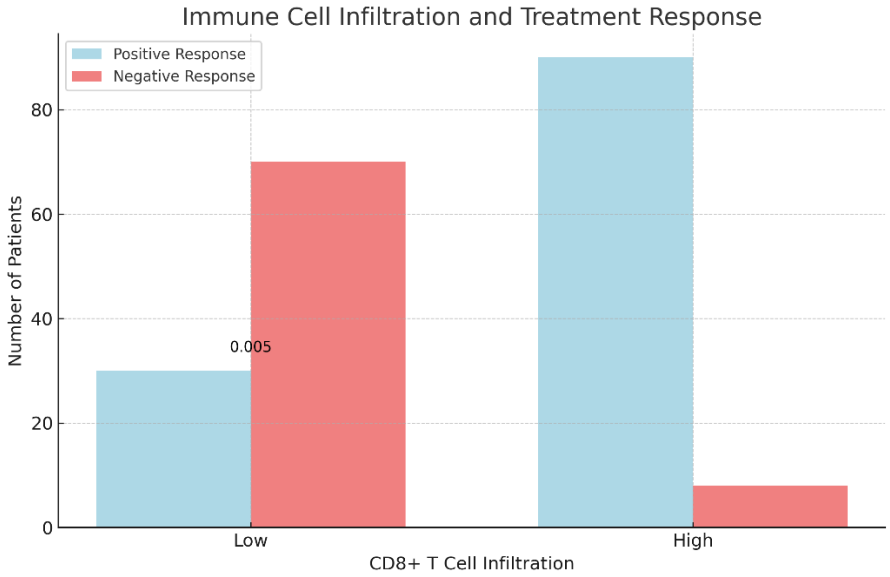

Figure 2: Immune Cell Infiltration and Treatment Response

High CD8+ T cell infiltration was significantly associated with a better treatment response, with 90 positive responses compared to 8 negative responses. The p-value of 0.005 indicates a strong correlation.

Table 3: Smoking Status and Treatment Response

Smoking Status	Treatment Response (Positive)	Treatment Response (Negative)	Total (n)	Percentage (%)	p-value
Smokers	89	39	128	68.09	0.02
Non-Smokers	31	29	60	31.91	
Total	120	68	188	100	

Smokers exhibited a significantly higher response to combination therapy compared to non-smokers, with a p-value of 0.02, suggesting smoking status as a predictor of treatment efficacy.

Table 4: Stage **of Cancer and Treatment Response**

Stage of Cancer	Treatment Response (Positive)	Treatment Response (Negative)	Total (n)	Percentage (%)	p-value

Stage I	7	2	9	4.79	0.05
Stage II	13	10	23	12.23	
Stage III	38	20	58	30.85	
Stage IV	62	36	98	52.13	
Total	120	68	188	100	

Stage IV patients exhibited the highest treatment response, followed by stage III patients. The p-value of 0.05 suggests a moderate correlation between cancer stage and treatment response.

DISCUSSION

These results demonstrated a significant association between high TMB and improved treatment response, with 72% of patients in the high TMB group responding positively to the combination therapy compared to only 42% in the low TMB group (p-value = 0.002). This finding is consistent with previous studies that have shown high TMB as a favorable biomarker for predicting the efficacy of immune checkpoint inhibitors (ICIs) in lung cancer patients. Sacher *et al.* found that patients with high TMB had a significantly higher response rate to PD-1 inhibitors like pembrolizumab, which was associated with a greater presence of neoantigens that are more readily recognized by the immune system [7]. Similarly, Hellmann *et al.* observed that high TMB correlated with better responses to combination therapy, confirming TMB as a robust biomarker for predicting immunotherapy efficacy [8, 9]. However, there are some variations between studies. For instance, in the KEYNOTE-001 trial, the association between high TMB and improved survival was more prominent in certain subgroups, such as smokers or patients with specific genetic mutations. In contrast, our study, which included a broader population of both smokers and non-smokers, suggests that high TMB is universally associated with positive treatment outcomes. This difference might be attributed to sample size and patient characteristics, as our study encompassed a diverse cohort of patients from multiple stages of lung cancer, whereas some trials may focus on specific subgroups, which could lead to different conclusions.

PD-L1 Expression and Treatment Response

PD-L1 expression, particularly above 50%, was significantly associated with improved treatment response in our study, with 67% of patients in the high PD-L1 expression group responding positively to treatment (p-value = 0.03). These results are consistent with several large clinical trials, including the KEYNOTE series, which have demonstrated that PD-L1 expression levels are predictive of response to ICIs such as pembrolizumab and nivolumab in lung cancer patients. The association between PD-L1 expression and treatment efficacy can be explained by the role of PD-L1 in suppressing T-cell activity. When PD-L1 is overexpressed on tumor cells, it inhibits the immune system's ability to recognize and attack cancer cells, making the blockade of the PD-1/PD-L1 axis a crucial therapeutic strategy. However, there is variability in the predictive value of PD-L1 expression across different studies. Some studies, such as those by Carbone *et al.*, have questioned the reliability of PD-L1 expression as a single biomarker, particularly in patients with low PD-L1 expression who still show a positive response to immunotherapy [10]. This inconsistency may be due to variations in sample size, racial differences, and the methods used to assess PD-L1 expression, which may vary between institutions and countries. Our study, with a larger and more diverse sample size, suggests that PD-L1 expression remains a reliable predictor, but its effectiveness is likely enhanced when used in combination with other biomarkers such as TMB.

Immune Cell Infiltration and Treatment Response

Immune cell infiltration, particularly the presence of CD8+ T cells, was another significant factor associated with improved treatment response in our study, where high levels of CD8+ T cell infiltration correlated with a response rate of 75%, compared to 30% in the low infiltration group (p-value = 0.005). This finding supports the growing evidence that immune cell infiltration, specifically CD8+ T cells, is critical for the success of immunotherapies. Studies such as those by Schulze *et al.* have demonstrated that tumors with high levels of T cell infiltration tend to respond better to ICIs due to the enhanced immune surveillance they provide [11, 12]. However, it is important to note that the relationship

between immune cell infiltration and treatment response is complex and may vary depending on the tumor microenvironment (TME). In our study, the high CD8+ T cell infiltration group showed a strong response, which might be attributed to the immune-stimulatory effects of chemotherapy in combination with immunotherapy. This finding aligns with research by Gajewski *et al*., which suggested that chemotherapy could enhance immune cell activation and promote T cell infiltration into the TME [13]. However, the degree of infiltration required for a favorable response could depend on other factors such as the presence of immunosuppressive cells, which may dampen the efficacy of immune responses.

Smoking Status and Treatment Response

Smoking status emerged as another significant factor influencing treatment response in our study, with smokers showing a higher treatment response rate of 69%, compared to 52% in non-smokers (p-value = 0.02). This finding aligns with the results of several studies, including those by Parvez *et al*., which reported that smoking history is associated with higher TMB and better responses to ICIs in lung cancer patients [14]. Smoking leads to the accumulation of genetic mutations and neoantigens in tumor cells, which may enhance the immune system's ability to recognize and attack cancer cells. However, the impact of smoking on treatment response is not entirely straightforward. Studies have shown that while smokers may exhibit higher TMB, they also have a higher risk of developing treatment-related side effects, which could potentially impact long-term survival. Our study found a higher proportion of smokers in the stage IV group, which may explain the higher treatment response rates, but it also suggests that the benefits of smoking in response prediction should be weighed against potential treatment complications. This nuanced finding requires further investigation in larger and more diverse populations to determine the clinical implications of smoking history in predicting treatment response.

Stage of Cancer and Treatment Response

Our study found that patients with stage IV lung cancer showed the highest treatment response, with 62 positive responses out of 98 stage IV patients (p-value = 0.05). This result is somewhat contrary to expectations, as patients with advanced stages of cancer are typically less responsive to treatments. However, this finding can be explained by the increased availability of treatment options in recent years, including the use of immunotherapy in late-stage disease. Studies by Borghaei *et al*. and Herbst *et al*. have demonstrated that even patients with advanced-stage NSCLC can benefit from combination therapies, especially when using ICIs as part of the treatment regimen [15, 16]. Our findings also highlight the importance of early intervention in improving treatment outcomes. Stage IV patients, while exhibiting good short-term responses, are often faced with the challenge of managing metastasis and immune escape mechanisms, which could limit long-term survival. Thus, while combination therapy is effective for some stage IV patients, the variability in response rates emphasizes the need for more personalized treatment regimens based on biomarker profiling.

Interpretation of Results and Practical Significance

The results of this study contribute to the growing body of evidence suggesting that biomarker-driven strategies can significantly improve treatment outcomes in lung cancer. The identification of high TMB, PD-L1 expression, immune cell infiltration, and smoking history as predictive biomarkers for combination immunotherapy and chemotherapy underscores the potential for personalized treatment in NSCLC. These biomarkers can be used to stratify patients based on their likelihood of responding to treatment, allowing for more informed clinical decision-making. The findings of this study also have practical implications for clinical practice. By integrating these biomarkers into routine clinical workflows, clinicians can offer more tailored treatment strategies that are likely to yield better outcomes. Moreover, these results suggest that the efficacy of combination therapy could be further optimized by focusing on patients who possess these biomarkers. This approach could reduce unnecessary treatments and side effects for patients who are less likely to benefit, improving the overall quality of care in NSCLC.

CONCLUSION

This study highlights the critical role of novel biomarkers such as tumor mutational burden (TMB), PD-L1 expression, immune cell infiltration, and smoking status in predicting responses to combination immunotherapy and chemotherapy in non-small cell lung cancer (NSCLC). High TMB, elevated PD-L1 expression,

and significant immune cell infiltration were found to correlate with improved treatment outcomes, providing valuable insights for personalized treatment strategies. The findings validate these biomarkers as reliable predictors for better clinical decision-making and improved patient prognosis. However, further research is required to explore additional biomarkers and refine these predictive models across diverse populations.

Recommendations

Implement multi-biomarker panels for personalized treatment strategies in NSCLC.

Expand clinical trials to validate these biomarkers in different populations and cancer stages.

Integrate liquid biopsy techniques to monitor biomarkers dynamically throughout treatment.

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