

Original scientific paper

## Pembrolizumab in PD-L1-positive advanced non-small cell lung carcinoma: A meta-analysis of survival benefits and immune-related toxicity events patterns

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### Abstract

**Background and objective:** Pembrolizumab has shown significant therapeutic benefit in advanced non-small cell lung cancer (NSCLC), but it remains uncertain which patients will benefit the most, and recent data suggest that programmed death-ligand 1 (PD-L1) expression as a single predictive biomarker is insufficient. This systematic review and meta-analysis looked at the safety and efficacy of pembrolizumab in PD-L1-positive advanced NSCLC patients, with a particular focus on disparities in treatment response to PD-L1 level of expression and demographic characteristics. **Method:** According to the PRISMA 2020 guidelines, six large databases were searched up to March 2025 for randomized controlled trials comparing pembrolizumab with chemotherapy in patients with such conditions. Overall survival (OS) and progression-free survival (PFS) were chosen as primary outcomes, and overall response rate (ORR) and safety profiles as secondary endpoints. A meta-analysis was conducted using a random-effects model, and the Cochrane risk of bias (ROB2) tool was employed to evaluate study quality. Seven randomized controlled trials involving 4,900 patients were included in the analysis. **Key results:** Pembrolizumab had a substantially better performance compared to chemotherapy for all the measures of efficacy: OS (hazard ratio (HR) 0.65, 95 % confidence interval (CI): 0.57 to 0.73,  $P < 0.00001$ ), PFS (HR 0.55, 95 % CI: 0.42 to 0.72,  $P < 0.0001$ ) and ORR (relative risk 2.10, 95 % CI: 1.51 to 2.93,  $P < 0.0001$ ). Subgroup analysis showed greater survival benefit in patients younger than 65 years (OS HR 0.55) compared to patients aged 65 and older (OS HR 0.72), and in females (OS HR 0.44) compared to males (OS HR 0.67). Of most significant importance, those with PD-L1 expression  $<1\%$  also saw considerable benefit in survival (OS HR 0.60), casting doubts over the existing biomarker-based selection criteria. **Conclusion:** In conclusion, pembrolizumab achieves clinically meaningful survival benefits and an acceptable toxicity in PD-L1-positive advanced NSCLC. The high efficacy observed even in low PD-L1 expressers, and demographic differences in drug response, suggest that existing patient selection criteria could potentially be extended. These findings justify the application of a more advanced approach involving multiple biomarkers for more precise treatment allocation.

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### Keywords

Immunotherapy; systematic review; biomarker, overall survival, progression-free survival, drug response

## Introduction

Lung cancer remains the leading cause of cancer-related deaths worldwide, with non-small cell lung cancer (NSCLC) accounting for roughly 85 % of all cases [1,2]. Over the past decade, treatment strategies for advanced NSCLC have shifted dramatically, moving from conventional chemotherapy toward personalized targeted therapies and immune checkpoint inhibitors (ICIs) [3-5]. These advances have translated into meaningful improvements in clinical outcomes, offering durable treatment responses and prolonged survival for patient groups that previously had very limited options [6,7].

Among immune checkpoint pathways, the programmed cell death protein-1 (PD-1) and its ligand PD-L1 have emerged as central therapeutic targets [8,9]. Their interaction functions as a key immune checkpoint, preventing excessive T-cell activation and maintaining immune balance [10]. However, tumour cells often exploit this mechanism by overexpressing PD-L1, thereby suppressing T-cell-mediated anti-tumour responses and escaping immune surveillance [11-13]. Blocking this pathway with monoclonal antibodies has proven effective in restoring immune-driven tumour destruction across multiple cancer types [14,15].

Pembrolizumab, a humanized IgG4-κ monoclonal antibody that targets PD-1, has demonstrated substantial clinical benefit in advanced NSCLC [16,17]. Results from the pivotal KEYNOTE trials consistently show improved Overall survival (OS) and progression-free survival (PFS) compared with standard chemotherapy, leading to its approval and widespread use in clinical practice [18-20]. These findings have established pembrolizumab as a cornerstone of first-line therapy for advanced NSCLC, fundamentally reshaping treatment guidelines and prognostic expectations [21,22].

Despite these advances, important challenges remain in refining patient selection for pembrolizumab. Currently, PD-L1 expression assessed by immunohistochemistry serves as the primary predictive biomarker, with tumour proportion scores (TPS)  $\geq 50$  % indicating the highest likelihood of response to monotherapy [23,24]. Yet, increasing evidence suggests that PD-L1 alone is insufficient for guiding treatment. Some patients with little or no PD-L1 expression achieve meaningful responses, while others with high expression levels derive limited benefit [25,26]. This paradox underscores the complexity of tumour-immune interactions, which cannot be fully explained by a single biomarker [27,28].

Clinical data also reveal wide variability in treatment outcomes across patient subgroups, indicating that demographic and molecular factors beyond PD-L1 play a role [29-31]. For instance, age and sex have been suggested as potential modifiers of immunotherapy efficacy, but these influences remain underexplored in systematic analyses [32-35]. In addition, long-term follow-up data from pivotal trials, along with emerging real-world evidence, are providing new insights into pembrolizumab's clinical profile that call for updated evaluation [36-38].

Although several meta-analyses have assessed pembrolizumab in NSCLC, most included unselected patient populations or did not focus specifically on PD-L1-positive disease using the most contemporary data [39,40]. A critical knowledge gap therefore persists regarding the true therapeutic value of pembrolizumab in patients with PD-L1-positive advanced NSCLC, particularly in light of evolving biomarker limitations and patient-related factors influencing treatment response [41,42].

To address this gap, we conducted a systematic review and meta-analysis to evaluate the efficacy and safety of pembrolizumab in PD-L1-positive advanced NSCLC, with a particular focus on clinical and molecular predictors of response. Our primary objective was to synthesize evidence from randomized controlled trials to assess survival outcomes across different PD-L1 expression thresholds and patient subgroups. Secondary objectives included a comprehensive evaluation of safety and an examination of demographic factors related

to treatment outcomes. Our analysis indicates that pembrolizumab significantly improves survival across all PD-L1 expression levels, with unexpected benefits in traditionally “biomarker-negative” patients and distinct response patterns linked to demographic characteristics. These findings challenge current patient selection paradigms and highlight the need for multidimensional biomarker strategies in the era of precision immunotherapy.

## Research design

A comprehensive literature search was conducted across multiple electronic databases, including PubMed/MEDLINE, Scopus, the Cochrane Library, Web of Science, ScienceDirect and EBSCO, covering publications from inception through March 2025. The search strategy combined Medical Subject Headings (MeSH) with free-text keywords such as “Non-Small Cell Lung Cancer”, “Pembrolizumab” and “PD-L1”, using Boolean operators (AND, OR) to ensure sensitivity and specificity. To minimize publication bias, additional gray literature sources were explored, including institutional repositories and relevant scientific platforms.

## Eligibility criteria

Studies were considered eligible if they met the following criteria: 1) Population: adults with PD-L1-positive NSCLC with histologic confirmation; 2) Intervention: pembrolizumab monotherapy or combination therapy in Phase II/III randomized controlled trials; 3) Control: chemotherapy; 4) Outcomes: OS, PFS, overall response rate (ORR), and safety outcomes; 5) Language: English-language publications.

Study selection was independently performed by two reviewers following a predefined protocol. Disagreements were resolved by discussion and, when necessary, consultation with a third reviewer.

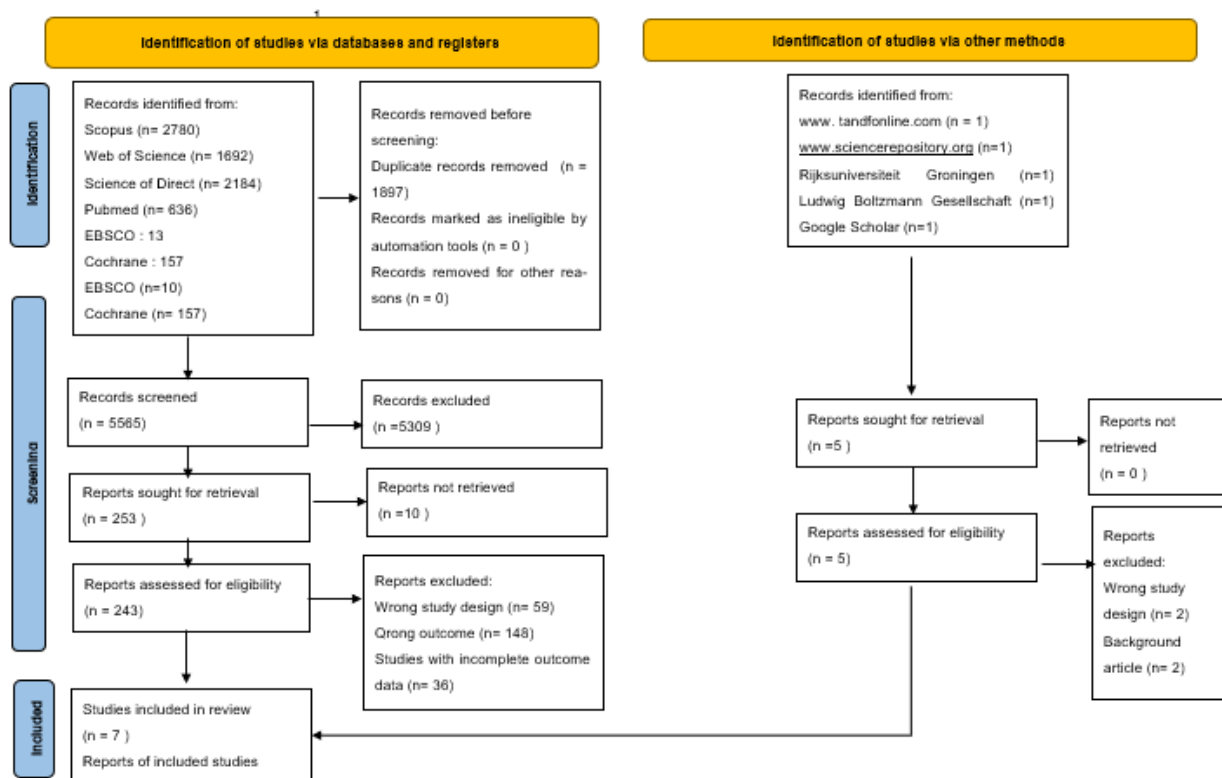
## Data analysis

Data were extracted on study design, patient demographics, intervention details, and clinical outcomes. The methodological quality of included studies was assessed using the revised Cochrane Risk of Bias tool (RoB 2), which evaluates five domains of potential bias. Statistical analyses were performed using Review Manager (RevMan) version 5.4. Hazard ratios (HRs) with 95 % confidence intervals (CIs) were used for time-to-event outcomes, Overall survival (OS) and progression-free survival (PFS), while relative risks were used for dichotomous outcomes (ORR and safety). Heterogeneity was assessed using the  $I^2$  statistic, which guided the choice between fixed- and random-effects models. Prespecified subgroup analyses were conducted according to PD-L1 expression levels and key patient characteristics.

## Results and discussion

### *Study selection and characteristics*

Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of literature screening and study selection is shown in Figure 1. The initial search identified 7,459 records, along with 5 additional records from other sources. After removing duplicates, 5,562 studies were screened, of which 7 randomized controlled trials (RCTs) published between 2016 and 2023 met the inclusion criteria, which are summarized in Table 1. In total, 4,900 patients were analysed, with 2,084 receiving pembrolizumab monotherapy and 1,816 assigned to chemotherapy as the control arm.



**Figure 1.** PRISMA flow diagram of literature screening and study selection

**Table 1.** Characteristics of included studies

| Ref. Year | Trial ID KEYNOTE | Phase | Treatment arms                                          | Total, n | Median follow-up, months | Sex, n / % |            | Histology, n / % |               | ECOG performance status, n / % |            | Smoking status, n / % |            | Geographic region, n / % |                      |            |
|-----------|------------------|-------|---------------------------------------------------------|----------|--------------------------|------------|------------|------------------|---------------|--------------------------------|------------|-----------------------|------------|--------------------------|----------------------|------------|
|           |                  |       |                                                         |          |                          | Male       | Female     | Squa-mous        | Non-squa-mous | 0                              | 1          | Current /former       | Never      | East Asia                | Europe North America | Other      |
| [43] 2016 | 010              | III   | Pembrolizumab 2 mg vs. 10 mg vs. Docetaxel              | 1,034    | 13.1                     | 634 (61 %) | 399 (39 %) | 222 (21 %)       | 724 (70 %)    | 348 (34 %)                     | 678 (66 %) | 833 (81 %)            | 190 (18 %) | 190 (18 %)               | 781 (76 %)           | 63 (6 %)   |
| [44] 2016 | 021              | II    | Pembrolizumab + Chemotherapy vs. Chemotherapy           | 123      | 10.6                     | 48 (39 %)  | 75 (61 %)  | 0 (0 %)          | 121 (98 %)    | 53 (43 %)                      | 69 (56 %)  | 99 (80 %)             | 24 (20 %)  | 0 (0 %)                  | 123 (100 %)          | 0 (0 %)    |
| [22] 2016 | 024              | III   | Pembrolizumab vs. Chemotherapy                          | 305      | 59.9                     | 187 (61 %) | 118 (39 %) | 56 (18 %)        | 249 (82 %)    | 107 (35 %)                     | 197 (65 %) | 280 (92 %)            | 24 (8 %)   | 40 (13 %)                | 265 (87 %)           | 0 (0 %)    |
| [45] 2019 | 042              | III   | Pembrolizumab vs. Chemotherapy                          | 1,274    | 12.8                     | 902 (71 %) | 372 (29 %) | 492 (39 %)       | 782 (61 %)    | 390 (31 %)                     | 884 (69 %) | 1,041 (82 %)          | 282 (22 %) | 370 (29 %)               | 286 (22 %)           | 618 (49 %) |
| [46] 2018 | 407              | III   | Pembrolizumab + Chemotherapy vs. Placebo + Chemotherapy | 559      | 7.8                      | 455 (81 %) | 104 (19 %) | 546 (98 %)       | 13 (2 %)      | 163 (29 %)                     | 396 (71 %) | 518 (93 %)            | 41 (7 %)   | 106 (19 %)               | 453 (81 %)           | 0 (0 %)    |
| [47] 2023 | 033              | III   | Pembrolizumab vs. Docetaxel                             | 425      | 22.3                     | 321 (76 %) | 104 (24 %) | 170 (40 %)       | 247 (58 %)    | 69 (16 %)                      | 356 (84 %) | 354 (83 %)            | 71 (17 %)  | 358 (84 %)               | 67 (16 %)            | 0 (0 %)    |
| [48] 2018 | 189              | III   | Pembrolizumab + Chemotherapy vs. Placebo + Chemotherapy | 616      | 10.5                     | 363 (59 %) | 253 (41 %) | 0 (0 %)          | 606 (98 %)    | 266 (43 %)                     | 346 (56 %) | 543 (88 %)            | 73 (12 %)  | 10 (2 %)                 | 531 (86 %)           | 75 (12 %)  |

### Risk of bias assessment

Risk of bias assessment using the Cochrane ROB2 tool determined that one study showed low risk across all domains. The majority of studies revealed "some concerns," primarily in Domain 2 (bias resulting from deviations from the planned intervention) and Domain 5 (bias introduced through the study's rollout), as shown in Figure 2. This pattern aligns with findings reported in other systematic reviews of immunotherapy.

Domain 2 problems were due to the following causes: 1) Open-label study designs that would influence patient/clinician behaviour; 2) High crossover rates from control to intervention group (range: 20.3 to 66 %); 3) Differential discontinuation patterns between treatment groups.



Figure 2. Assessment of risk of bias

Domain 5 concerns were related to protocol amendments, post-hoc subgroup analyses, and selective outcome reporting. These issues, although consistent with broader challenges in immunotherapy research, did not significantly undermine the overall quality of the evidence.

Primary efficacy outcomes

Objective response rate

All seven trials reported ORR for PD-L1-positive tumours. Pembrolizumab doubled the likelihood of objective response compared with chemotherapy (RR 2.10, 95 % CI 1.51-2.93;  $P < 0.0001$ ), with 404 responses among 931 pembrolizumab patients versus 209 among 876 control patients (Figure 3A). Considerable heterogeneity ( $I^2 = 78\%$ ,  $P = 0.0001$ ) likely reflected variation in PD-L1 thresholds and patient populations across studies.

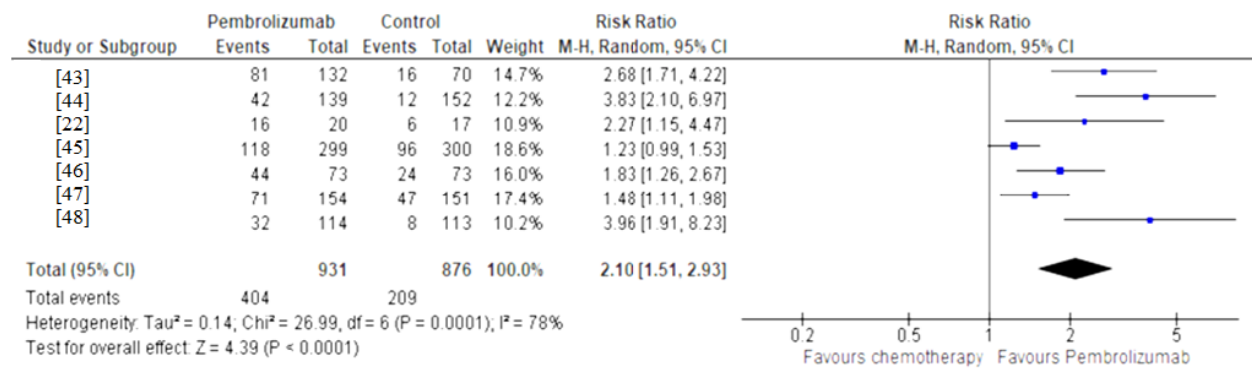
Total survival

Six studies provided OS data for the PD-L1-positive group. Pembrolizumab monotherapy achieved a clinically important 35 % reduction in hazard of death compared with chemotherapy (HR 0.65, 95 % CI: 0.57-0.73,  $P < 0.00001$ ) as observed in Figure 3B. The analysis showed low to moderate heterogeneity ( $I^2 = 30\%$ ,  $P = 0.21$ ), favouring homogeneous survival benefits across study populations. This is a notable clinical benefit with robust statistical significance.

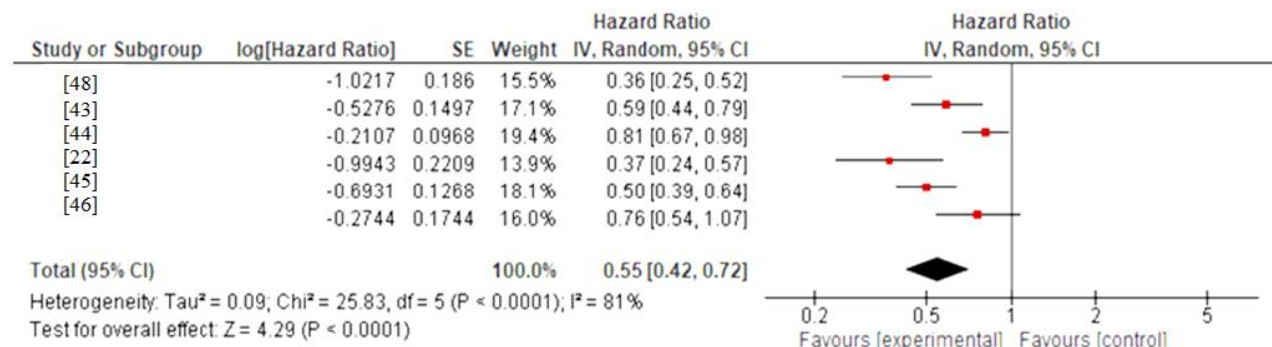
Progression-free survival

PFS analysis of six studies demonstrated that pembrolizumab significantly reduced the risk of disease progression or death by 45 % (HR 0.55, 95 % CI: 0.42-0.72,  $P < 0.0001$ ) as illustrated in Figure 3C. However, high heterogeneity was observed ( $I^2 = 81\%$ ,  $P < 0.00001$ ), suggesting variability in progression patterns across different patient populations and study designs. Despite this heterogeneity, the substantial effect size and statistical significance support the superior efficacy of pembrolizumab in delaying disease progression.

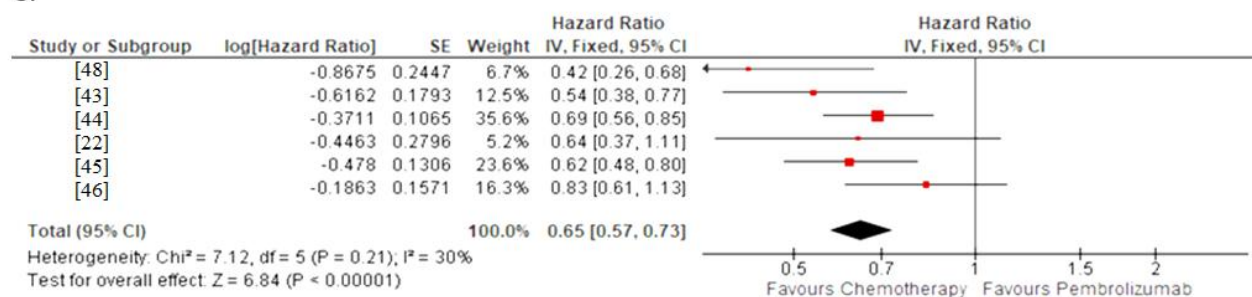
A.



B.



C.



**Figure 3.** Forest plots illustrating survival outcomes in randomized clinical trials (RCTs): (A) ORR in RCTs, (B) OS in RCTs and (C) PFS in RCTs

### Secondary outcomes

#### Safety analysis

In the combined safety analysis, 4,168 patients in the pembrolizumab arm and 3,632 patients in the control arm were included in seven randomized trials. Most patients in both arms experienced adverse events of any grade, with pembrolizumab having an evident but manageable safety profile compared to chemotherapy controls, as summarized in Table 2.

#### Overall safety profile

Grade 3-5 adverse events had variable incidence between trials, with severe toxicity of 11.3 to 67.2 % in patients treated with pembrolizumab, compared with 25.8 to 68.2 % in control arms, as summarized in Table 2. Treatment discontinuation due to adverse events was observed in pembrolizumab-treated patients at frequencies ranging from 4.4% to 13.6%, compared with frequencies ranging from 6.4 to 12.7 % in the control arms. Treatment-related mortality remained infrequent in both groups:

- Pembrolizumab: 0.9 to 6.6 % across trials,
- Control: 1.5 to 13.1 %,
- No overall mortality difference by treatment groups with pooled analysis.

**Table 2.** Overall safety summary and treatment-related adverse events

| Ref. | Treatment arms        | Total, n | Any grade AEs, n / % | Grade 3-5 AEs, n / % | Treatment-related deaths, n / % | Discontinuation due to AEs, n / % |
|------|-----------------------|----------|----------------------|----------------------|---------------------------------|-----------------------------------|
| [43] | Pembrolizumab 2 mg    | 344      | 215/339 (63.4 %)     | 43/339 (12.7 %)      | 3/344 (0.9 %)                   | 15/344 (4.4 %)                    |
|      | Docetaxel             | 343      | 251/309 (81.2 %)     | 109/309 (35.3 %)     | 5/343 (1.5 %)                   | 31/343 (9.0 %)                    |
| [44] | Pembrolizumab + Chemo | 60       | 55/59 (93.2 %)       | 23/59 (39.0 %)       | 1/60 (1.7 %)                    | 6/60 (10.0 %)                     |
|      | Chemotherapy          | 63       | 56/62 (90.3 %)       | 16/62 (25.8 %)       | 2/63 (3.2 %)                    | 8/63 (12.7 %)                     |
| [22] | Pembrolizumab         | 154      | 118/154 (76.6 %)     | 48/154 (31.2 %)      | 2/154 (1.3 %)                   | 21/154 (13.6 %)                   |
|      | Chemotherapy          | 151      | 135/150 (90.0 %)     | 80/150 (53.3 %)      | 3/151 (2.0 %)                   | 16/151 (10.6 %)                   |
| [45] | Pembrolizumab         | 637      | 399/636 (62.7 %)     | 113/636 (17.8 %)     | 13/637 (2.0 %)                  | 57/637 (8.9 %)                    |
|      | Chemotherapy          | 637      | 553/615 (89.9 %)     | 252/615 (41.0 %)     | 14/637 (2.2 %)                  | 58/637 (9.1 %)                    |
| [46] | Pembrolizumab + Chemo | 278      | 273/278 (98.2 %)     | 194/278 (69.8 %)     | 10/278 (3.6 %)                  | 37/278 (13.3 %)                   |
|      | Placebo + Chemo       | 281      | 274/280 (97.9 %)     | 191/280 (68.2 %)     | 6/281 (2.1 %)                   | 18/281 (6.4 %)                    |
| [47] | Pembrolizumab         | 213      | 149/213 (70.0 %)     | 24/213 (11.3 %)      | 4/213 (1.9 %)                   | 21/213 (9.9 %)                    |
|      | Docetaxel             | 212      | 174/198 (87.9 %)     | 94/198 (47.5 %)      | 4/212 (1.9 %)                   | 15/212 (7.1 %)                    |
| [48] | Pembrolizumab + Chemo | 410      | 404/405 (99.8 %)     | 272/405 (67.2 %)     | 27/410 (6.6 %)                  | NR                                |
|      | Placebo + Chemo       | 206      | 200/202 (99.0 %)     | 133/202 (65.8 %)     | 27/206 (13.1 %)                 | NR                                |

*Immune-related adverse events*

Immune-mediated adverse events of any grade occurred more frequently in pembrolizumab-treated patients compared to controls, with incidence rates ranging from 20.1 to 34.4 % versus 5.3 to 11.3 % in control groups. Grade 3-5 immune-related adverse events showed rates of 5.1 to 13.6 % in pembrolizumab patients compared to 0.7 to 3.2 % in controls.

Pneumonitis emerged as the most clinically significant immune-related adverse event (see Table 3): any grade: 4.0 to 9.9 % in pembrolizumab patients versus 0 to 2.5 % in controls; Grade 3-5: 1.7 to 3.2 % versus 0 to 2.0 % in control groups. Endocrine toxicities demonstrated characteristic patterns: Hypothyroidism: 7.9 to 15.3 % in pembrolizumab versus 0.3 to 11.9 % in controls; Grade 3-5 hypothyroidism: 0 to 1.0 % versus 0 to 0.5 % in controls; Hyperthyroidism: 4.0 to 8.0 % versus 0 to 3.0 % in controls.

**Table 3.** Immune-mediated adverse events

| Ref. | Treatment arms        | Total, n | Any grade irAEs, n / % | Grade 3-5 irAEs, n / % | Most common irAEs (≥5 % incidence)                                                     |
|------|-----------------------|----------|------------------------|------------------------|----------------------------------------------------------------------------------------|
| [43] | Pembrolizumab, 2 mg   | 344      | 69/344 (20.1 %)        | NR                     | Hypothyroidism (8 %), hyperthyroidism (4 %), pneumonitis (4 %)                         |
|      | Docetaxel             | 343      | NR                     | NR                     | Minimal immune-related events                                                          |
| [44] | Pembrolizumab + Chemo | 60       | 13/59 (22.0 %)         | 3/59 (5.1 %)           | Hypothyroidism (15 %), hyperthyroidism (8 %), pneumonitis (5 %)                        |
|      | Chemotherapy          | 63       | 7/62 (11.3 %)          | 0/62 (0 %)             | Minimal immune-related events                                                          |
| [22] | Pembrolizumab         | 154      | 53/154 (34.4 %)        | 21/154 (13.6 %)        | Hypothyroidism (10.4 %), hyperthyroidism (7.1 %), pneumonitis (8.4 %)                  |
|      | Chemotherapy          | 151      | 8/150 (5.3 %)          | 1/150 (0.7 %)          | Minimal immune-related events                                                          |
| [45] | Pembrolizumab         | 637      | 177/636 (27.8 %)       | 51/636 (8.0 %)         | Pneumonitis (22 patients), severe skin reactions (11 patients), hepatitis (7 patients) |
|      | Chemotherapy          | 637      | 44/615 (7.2 %)         | 9/615 (1.5 %)          | Minimal immune-related events                                                          |
| [46] | Pembrolizumab + Chemo | 278      | 80/278 (28.8 %)        | 30/278 (10.8 %)        | Hypothyroidism (7.9 %), pneumonitis (6.5 %), colitis (2.5 %)                           |
|      | Placebo + Chemo       | 281      | 24/280 (8.6 %)         | 9/280 (3.2 %)          | Minimal immune-related events                                                          |
| [47] | Pembrolizumab         | 213      | 61/213 (28.6 %)        | 13/213 (6.1 %)         | Hypothyroidism (13.1 %), pneumonitis (9.9 %), hepatitis (1.9 %)                        |
|      | Docetaxel             | 212      | 12/198 (6.1 %)         | 3/198 (1.5 %)          | Minimal immune-related events                                                          |
| [48] | Pembrolizumab + Chemo | 410      | NR                     | NR                     | NR                                                                                     |
|      | Placebo + Chemo       | 206      | NR                     | NR                     | NR                                                                                     |

### *Specific adverse event profiles*

Hematologic toxicities revealed significant differences favouring pembrolizumab: Neutropenia occurred substantially less frequently in pembrolizumab patients (0 to 38 %) compared to chemotherapy controls (6 to 24 %); Grade 3-5 neutropenia showed even more pronounced differences (pembrolizumab: 0 to 23 %; control: 2 to 25 %); Similar patterns were observed for anemia and other hematologic parameters.

Gastrointestinal toxicities showed divergent patterns: Nausea occurred with variable frequency in pembrolizumab patients (1 to 56 %) compared to chemotherapy controls (13 to 52 %); Diarrhea demonstrated variable incidence (pembrolizumab: 2 to 31 %; control: 7 to 23 %); Immune-mediated colitis occurred in 0.6 to 3.9 % of pembrolizumab patients compared to 0 % to 1.4 % in controls. The safety analysis confirmed pembrolizumab's distinct toxicity profile, characterized by immune-related adverse events while demonstrating reduced incidence of traditional chemotherapy-related toxicities. This differential toxicity profile supports the clinical utility of pembrolizumab, particularly in patients who may not tolerate intensive chemotherapy regimens.

### *Subgroup analyses*

#### *Age-stratified analysis*

Age had a consistent and significant impact on pembrolizumab effectiveness in both survival endpoints, as analysed by PFS and OS, as demonstrated by Tables 4 and 5, respectively. In OS, patients younger than 65 years received significantly more benefit (HR 0.55; 95 % CI 0.46 to 0.65;  $P < 0.00001$ ) compared to patients 65 years or older (HR 0.72; 95 % CI 0.59 to 0.88;  $P = 0.001$ ). This age-related trend was also evident in the PFS analysis, with the younger group showing a significantly greater benefit (HR 0.46; 95 % CI 0.38-0.57;  $P < 0.00001$ ) compared to older patients (HR 0.68; 95 % CI 0.55 to 0.85;  $P = 0.0005$ ).

Heterogeneity was low for the young population for OS ( $I^2 = 44$  %) and zero for PFS ( $I^2 = 0$  %), while older patients demonstrated no heterogeneity across studies ( $I^2 = 0$  % for both endpoints), supporting strong and consistent age-related treatment effects.

#### *Sex-based analysis*

Gender stratification revealed dramatic differences in pembrolizumab efficacy magnitude among endpoints. In the OS of the PD-L1  $\geq 50$  % subgroup, female patients exhibited exceptional benefit (HR 0.44; 95 % CI 0.24-0.82;  $P = 0.009$ ), significantly better than that of male patients (HR 0.67; 95 % CI 0.57-0.79;  $P < 0.00001$ ). The female OS analysis revealed, nonetheless, significant heterogeneity ( $I^2 = 87$  %), reflecting heterogeneity of response to treatment across studies.

This sex difference occurred uniformly in PFS, where females fared better (HR 0.43; 95 % CI 0.32-0.56;  $P < 0.00001$ ) than males (HR 0.61; 95 % CI 0.51-0.73;  $P < 0.00001$ ) (Tables 2 and 3). Notably, the PFS analysis did not reveal any heterogeneity within either sex stratum ( $I^2 = 0$  %), indicating consistent treatment effects within each gender group.

#### *Performance status analysis*

Eastern Cooperative Oncology Group (ECOG) performance status indicated pembrolizumab efficacy across all functional status levels with relatively small variation between groups. OS analysis indicated significant benefit in both ECOG 0 (HR 0.60; 95 % CI 0.47 to 0.77;  $P < 0.0001$ ) and ECOG 1 patients (HR 0.66; 95 % CI 0.57-0.77;  $P < 0.00001$ ). The ECOG 0 subgroup demonstrated moderate heterogeneity ( $I^2 = 38$  %), while ECOG 1 patients demonstrated total homogeneity ( $I^2 = 0$  %).

PFS also concurred with results in ECOG 0 patients HR 0.47 (95 % CI 0.36 to 0.62;  $P < 0.00001$ ) and ECOG 1 patients HR 0.59 (95 % CI 0.49 to 0.70;  $P < 0.00001$ ) (Tables 2 and 3). Notably, both PFS subgroups did not

have heterogeneity ( $I^2 = 0\%$ ), implying extremely consistent treatment effects across the varying baseline functional status.

#### PD-L1 expression analysis

The stratification of tumour score proportion has demonstrated the efficacy of pembrolizumab across the full spectrum of PD-L1 levels, revealing unexpected trends that contradict traditional biomarker-driven selection frameworks. OS and PFS analyses in Tables 4 and 5, respectively, further confirmed that patients with a TPS of less than 1 % experienced significant benefits, indicated by a hazard ratio of 0.60 (95 % CI 0.43 to 0.83;  $P = 0.002$ ). In contrast, those with TPS ranging from 1 to 49 % exhibited similar efficacy, with a hazard ratio of 0.56 (95 % CI 0.40 to 0.78;  $P=0.0006$ ).

**Table 4.** Analysis of OS in patient subgroups with varying clinical characteristics

| Population                  | Subgroup | No. of studies | HR   | 95 % CI   | $I^2$ / % | $p$ value |
|-----------------------------|----------|----------------|------|-----------|-----------|-----------|
| Age-Stratified Analysis     |          |                |      |           |           |           |
| Age < 65 years              | Total    | 3              | 0.55 | 0.46-0.65 | 44        | <0.00001  |
| Age ≥ 65 years              | Total    | 3              | 0.72 | 0.59-0.88 | 0         | 0.001     |
| Sex-Based Analysis          |          |                |      |           |           |           |
| Male                        | Total    | 3              | 0.67 | 0.57-0.79 | 0         | <0.00001  |
| Female (PD-L1 ≥50 %)        | Total    | 3              | 0.44 | 0.24-0.82 | 87        | 0.009     |
| Performance Status Analysis |          |                |      |           |           |           |
| ECOG PS 0                   | Total    | 3              | 0.60 | 0.47-0.77 | 38        | <0.0001   |
| ECOG PS 1                   | Total    | 3              | 0.66 | 0.57-0.77 | 0         | <0.00001  |
| PD-L1 Expression Analysis   |          |                |      |           |           |           |
| PD-L1 TPS < 1 %             | Total    | 2              | 0.60 | 0.43-0.83 | 0         | 0.002     |
| PD-L1 TPS ≥ 1 %             | Total    | 5              | 0.71 | 0.60-0.84 | 59        | <0.0001   |
| PD-L1 TPS 1-49 %            | Total    | 2              | 0.56 | 0.40-0.78 | 0         | 0.0006    |

Patients classified with TPS of 1 % or greater showed a significant, albeit less pronounced, benefit, reflected by a hazard ratio of 0.71 (95 % CI 0.60 to 0.84;  $P < 0.0001$ ), accompanied by moderate heterogeneity ( $I^2=59\%$ ). In the cohort of patients with TPS less than 1 %, the analysis of progression-free survival indicated a consistent benefit, with a hazard ratio of 0.60 (95 % CI 0.43 to 0.83;  $P = 0.002$ ). Conversely, patients with TPS between 1 and 49 % demonstrated comparable efficacy, with a hazard ratio of 0.56 (95 % CI 0.43-0.73;  $P < 0.0001$ ).

**Table 5.** Analysis of PFS in patient subgroups with varying clinical characteristics

| Population                  | Subgroup | No. of studies | HR   | 95 % CI   | $I^2$ / % | $p$ value |
|-----------------------------|----------|----------------|------|-----------|-----------|-----------|
| Age-stratified analysis     |          |                |      |           |           |           |
| Age < 65 years              | Total    | 2              | 0.46 | 0.38-0.57 | 0         | <0.00001  |
| Age ≥ 65 years              | Total    | 2              | 0.68 | 0.55-0.85 | 0         | 0.0005    |
| Sex-based analysis          |          |                |      |           |           |           |
| Male                        | Total    | 2              | 0.61 | 0.51-0.73 | 0         | <0.00001  |
| Female                      | Total    | 2              | 0.43 | 0.32-0.56 | 0         | <0.00001  |
| Performance status analysis |          |                |      |           |           |           |
| ECOG PS 0                   | Total    | 2              | 0.47 | 0.36-0.62 | 0         | <0.00001  |
| ECOG PS 1                   | Total    | 2              | 0.59 | 0.49-0.70 | 0         | <0.00001  |
| PD-L1 expression analysis   |          |                |      |           |           |           |
| PD-L1 TPS < 1 %             | Total    | 2              | 0.60 | 0.43-0.83 | %         | 0.002     |
| PD-L1 TPS ≥ 1 %             | Total    | 5              | 0.74 | 0.54-1.01 | 90        | 0.05      |
| PD-L1 TPS 1-49 %            | Total    | 2              | 0.56 | 0.43-0.73 | 0         | <0.0001   |

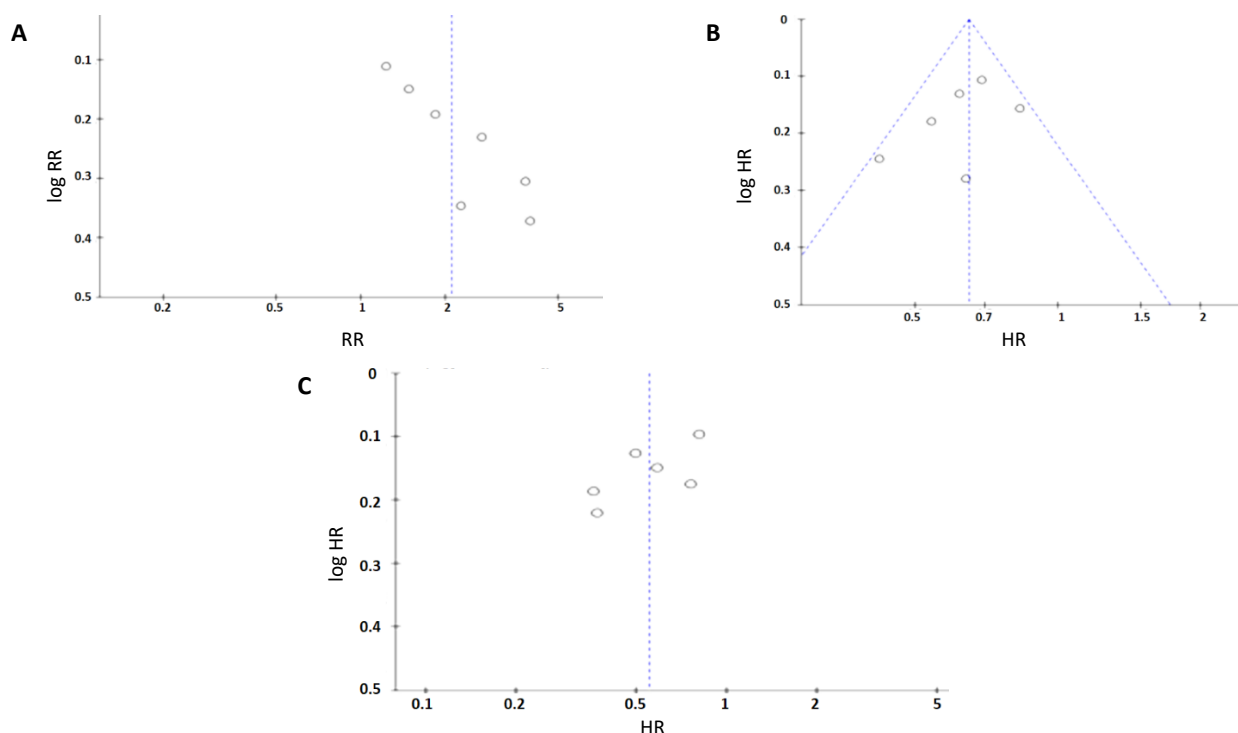
The subgroup of patients with TPS of 1 % or more exhibited only marginal statistical significance, as evidenced by a hazard ratio of 0.74 (95 % CI 0.54 to 1.01;  $P = 0.05$ ), alongside a notably high level of heterogeneity ( $I^2=90\%$ ). This surprising finding raises questions about established PD-L1-based selection criteria and highlights the need for further exploration of alternative biomarker approaches. The consistent benefit observed across various PD-L1 expression levels, particularly within the historically regarded

"biomarker-negative" group (TPS <1 %), advocates for a broader consideration of therapeutic options beyond the existing PD-L1-centric selection paradigms.

#### *Sensitivity analysis and publication bias*

Sensitivity analyses were conducted by systematically removing each study individually, which revealed that the primary outcomes remained consistent throughout these exclusions. This consistency demonstrates minimal sensitivity and reinforces the reliability and stability of the findings. The prognostic factors in the overall study population remained unaffected by these sensitivity analyses.

Assessment of publication bias showed no substantial bias, as indicated by the symmetrical funnel plots for both OS and PFS (Figure 4), as well as for the subgroup analyses of these endpoints.



**Figure 4.** Funnel plots for (A) ORR (B) OS (C) PFS between pembrolizumab and chemotherapy

These results strengthen the validity of the meta-analysis conclusions and suggest that the treatment effects observed are robust and not substantially influenced by selective publication practices.

## **Discussion**

### *Primary findings in context of previous research*

A pooled analysis revealed a 45 % reduction in the risk of disease progression with pembrolizumab (HR 0.55, 95 % CI: 0.42-0.72). This effect size was not only consistent with but, in some cases, stronger than results from individual trials such as KEYNOTE-024 (HR 0.50, 95 % CI: 0.37 to 0.68) and KEYNOTE-042 (HR 0.81, 95 % CI: 0.71 to 0.93) [46]. The twofold increase in objective response rate (RR 2.10, 95 % CI: 1.51 to 2.93) further illustrates pembrolizumab's potent antitumor activity, aligning with the established mechanism of checkpoint blockade that restores T-cell-mediated immune surveillance [49].

One of the most unexpected findings was that PD-L1 expression did not consistently predict treatment response. Patients with a TPS below 1%, typically considered "biomarker-negative," derived a clear survival benefit (OS HR 0.60, 95% CI: 0.43 to 0.83), in some instances comparable to those with higher PD-L1 expression [50]. This challenges the prevailing paradigm established by early pembrolizumab trials, which

restricted monotherapy to PD-L1-high populations (TPS  $\geq 50\%$ ) and still guides FDA approvals [51]. Our results support accumulating real-world evidence showing that PD-L1 alone cannot capture the complexity of tumour-immune interactions that shape responses to immunotherapy [52].

Age and sex also emerged as significant modifiers of treatment effect. Younger patients (<65 years) experienced substantially greater benefit than older patients (OS HR 0.55 vs. 0.72), suggesting immune senescence may blunt checkpoint inhibitor efficacy. This aligns with evidence of reduced T-cell function and immune surveillance with aging [53]. Similarly, women demonstrated greater survival benefit (OS HR 0.44 vs. 0.67 in men), consistent with the known sexual dimorphism in immune responses [54]. These findings suggest that demographic factors, often overlooked in patient selection, may meaningfully shape outcomes and should be considered alongside molecular biomarkers.

The most clinically significant implication of our study is the strong efficacy signal in patients with low or negative PD-L1 expression, a population currently excluded from pembrolizumab monotherapy under most treatment guidelines. Extending eligibility to this group could expand therapeutic options for up to 30 to 40 % of advanced NSCLC patients who are currently treated with chemotherapy alone [55].

The biological basis of this PD-L1 paradox is likely multifactorial, involving intratumoral heterogeneity, temporal variation in PD-L1 expression, and inflammation-induced changes during therapy [56]. Other contributors may include immune cell PD-L1 expression and additional checkpoint pathways [57]. Together, these factors highlight the limitations of PD-L1 as a standalone biomarker.

Our subgroup analyses provide biologically plausible insights that have immediate clinical relevance. Younger patients' stronger immune systems likely underpin their improved responses [58], whereas immune senescence in older individuals - characterized by thymic atrophy, T-cell exhaustion, and chronic inflammation - may diminish benefit [59]. Likewise, women's more active immune responses, while predisposing them to autoimmunity, may increase sensitivity to checkpoint blockade [60]. These differences emphasize the need for treatment strategies that account for patient demographics as well as tumour biology.

Safety findings were consistent with prior reports. Pembrolizumab was associated with immune-related toxicities such as pneumonitis (4 to 9.9 %) and hypothyroidism (7.9 to 15.3 %), but these events were generally manageable. Importantly, pembrolizumab carried a markedly lower risk of hematologic toxicities such as severe neutropenia (0 to 23 % vs. 2 to 25 % with chemotherapy) [61]. This favourable safety profile is particularly relevant for older patients, those with comorbidities, or individuals with poor performance status who may not tolerate chemotherapy.

The high heterogeneity observed in PFS analysis reflects differences in trial populations, PD-L1 assays, and comparator regimens. While this reduces precision, it increases generalizability by better reflecting real-world variation [62]. Still, reliance on trial populations limits applicability to routine practice, where patients often have greater comorbidity and lower performance status. The geographic underrepresentation of Asian populations further limits the external validity [63].

Our findings underscore the urgent need for more sophisticated biomarkers beyond PD-L1. Composite approaches incorporating tumour mutational burden, immune gene signatures, circulating biomarkers, and AI-driven imaging hold promise [64]. Advances in liquid biopsy technology, including circulating tumour DNA and immune profiling, could enable real-time monitoring of treatment response [65].

Although our analysis focused on pembrolizumab monotherapy, future progress will likely depend on rational combination strategies with chemotherapy, anti-angiogenic agents, and novel immunomodulators [66]. The observed demographic differences suggest tailoring these combinations to patient subgroups may

maximize benefit [67]. Integrating real-world evidence, registry data, and pragmatic trials will be crucial to understanding long-term outcomes and optimizing sequencing in diverse patient populations [68].

Taken together, our results signal a paradigm shift away from reliance on a single biomarker toward multifactorial patient selection models. Incorporating demographic, clinical, and molecular variables into integrated risk prediction frameworks could enhance precision in treatment decisions [69]. Machine learning and AI offer powerful tools to synthesize these diverse data sources and may reveal novel signatures of response that are not captured by traditional analyses [70].

From a regulatory and clinical practice perspective, the clear benefits observed in PD-L1-low patients raise the possibility of broadening pembrolizumab monotherapy indications. Such a shift will require balancing clinical efficacy with cost, health system capacity, and equity of access. Collaborative efforts among regulators, professional societies, and healthcare systems will be crucial to translating these insights into enhanced patient care.

Finally, our study has limitations. Reliance on trial-level rather than individual patient data constrained our ability to explore confounding factors and limited biomarker analyses, such as tumour mutational burden, which was inconsistently reported. These gaps highlight the importance of ongoing research to refine patient selection and therapeutic strategies.

## Conclusions

This meta-analysis provides robust evidence that pembrolizumab significantly improves overall survival, progression-free survival, and objective response rates in patients with advanced non-small cell lung cancer exhibiting PD-L1-positive expression. The treatment exhibits a tolerable safety profile, primarily characterized by immune-related adverse events rather than the typical cytotoxic side effects. Notably, the subgroup analyses presented here suggest that pembrolizumab offers considerable clinical benefits not only to patients with high PD-L1 expression but also to those with low or even undetectable PD-L1 levels—this finding challenges the established paradigms of biomarker-driven treatment. The variability in treatment responses among different age and gender cohorts highlights the biological diversity in patient reactions to immunotherapy and reinforces the need for developing more personalized therapeutic strategies. These findings underscore the limitations of PD-L1 expression as a solitary predictive biomarker and stress the importance of incorporating additional testing methods, such as tumour mutational burden, immune gene profiling, and liquid biopsy technologies into clinical practice. Overall, while pembrolizumab remains a cornerstone treatment for advanced NSCLC, optimizing patient selection through multidimensional biomarker strategies will be crucial to enhance therapeutic outcomes and accelerate personalized cancer treatment in the immunotherapy era.

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## References

- [1] N. Duma, R. Santana-Davila, J.R. Molina. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clinic Proceedings* **94** (2019) 1623-1640.  
<https://doi.org/10.1016/j.mayocp.2019.01.013>
- [2] P. Garg, S. Singhal, P. Kulkarni, D. Horne, J. Malhotra, R. Salgia, S. Singhal. Advances in Non-Small Cell Lung Cancer: Current Insights and Future Directions. *Journal of Clinical Medicine* **13** (2024) 45-56.  
<https://doi.org/10.3390/jcm13144189>

- [3] Q. Huang, Y. Li, Y. Huang, J. Wu, W. Bao, C. Xue, S. Hu. Advances in molecular pathology and therapy of non-small cell lung cancer. *Signal Transduction and Targeted Therapy* **10** (2025) 186-195. <https://doi.org/10.1038/s41392-025-02243-6>
- [4] M. Dawood M. Assi. Evaluation of her 2, and ki-67 expression immunohistochemically of gastric cancer in Al-Najaf province. *Procedia Environmental Science Engineering and Management*. **11** (2024) 465-472. [https://www.procedia-esem.eu/pdf/issues/2024/no3/46\\_Dawood\\_24.pdf](https://www.procedia-esem.eu/pdf/issues/2024/no3/46_Dawood_24.pdf)
- [5] H. Mamdani, S. Matosevic, A. B. Khalid, G. Durm, S. I. Jalal. Immunotherapy in Lung Cancer: Current Landscape and Future Directions. *Frontiers in Immunology* **13** (2022) 823618. <https://doi.org/10.3389/fimmu.2022.823618>
- [6] H. E. Marei, A. Hasan, G. Pozzoli, C. Cenciarelli. Cancer immunotherapy with immune checkpoint inhibitors (ICIs): potential, mechanisms of resistance, and strategies for reinvigorating T cell responsiveness when resistance is acquired. *Cancer Cell International* **23** (2023) 64-76. <https://doi.org/10.1186/s12935-023-02902-0>
- [7] M. F. Sanmamed, L. Chen. A paradigm shift in cancer immunotherapy: from enhancement to normalization. *Cell* **175** (2018) 313-326. <https://doi.org/10.1016/j.cell.2018.09.035>
- [8] A. Abaza, F. Idris, H. Shaikh, I. Vahora, K. Moparthi, M. Al Rushaidi, M. Muddam. Programmed Cell Death Protein 1 (PD-1) and Programmed Cell Death Ligand 1 (PD-L1) Immunotherapy: A Promising Breakthrough in Cancer Therapeutics. *Cureus Journal of Medical Science* **1** (2023) e44582. <https://doi.org/10.7759/cureus.44582>
- [9] Y. Shindo, S. Hazama, R. Tsunedomi, N. Suzuki, H. Nagano, Novel biomarkers for personalized cancer immunotherapy, *Cancers (Basel)* **11** (2019) 1223. <https://doi.org/10.3390/cancers11091223>
- [10] L. V. Mejía-Guarnizo, P. S. Monroy-Camacho, A. D. Turizo-Smith, J. A. Rodríguez-García. The role of immune checkpoints in antitumor response: a potential antitumor immunotherapy. *Frontiers in Immunology* **14** (2023) 1298571. <https://doi.org/10.3389/fimmu.2023.1298571>
- [11] D. Lee, M. Cho, E. Kim, Y. Seo, J. H. Cha. PD-L1: From cancer immunotherapy to therapeutic implications in multiple disorders. *Molecular Therapy* **32** (2024) 4235-4255. <https://doi.org/10.1016/j.ymthe.2024.09.026>
- [12] A. Mohammed, A. Al-Gawhari. Bioavailability enhancement techniques, and in vitro-in vivo evaluation of rosvastatin calcium-cyclodextrin inclusion complex nanofibers, *Procedia Environmental Science Engineering and Management*. **11** (2024) 117-134. [https://www.procedia-esem.eu/pdf/issues/2024/no1/13\\_Mohammed\\_24.pdf](https://www.procedia-esem.eu/pdf/issues/2024/no1/13_Mohammed_24.pdf)
- [13] J. H. Cha, L. C. Chan, C. W. Li, J. L. Hsu, M. C. Hung. Mechanisms Controlling PD-L1 Expression in Cancer. *Molecular Cell* **76** (2019) 359-370. <https://doi.org/10.1016/j.molcel.2019.09.030>
- [14] P. G. Abrams R. K. Oldham. Monoclonal Antibody Therapy of Solid Tumors. *Monoclonal Antibodies in Oral Cancer Therapy* **3** (1985) 103-120. [https://doi.org/10.1007/978-1-4613-2627-4\\_5](https://doi.org/10.1007/978-1-4613-2627-4_5)
- [15] Yuliana, I. Maulana, M. Prenggono, I. Oktavianti, N. Kania, R. Panghiyangani, N. Hasanah. Mercury exposure triggers diabetes mellitus through a pancreatic beta cell endoplasmic reticulum stress mechanism. *Revista Latinoamericana de Hipertension* **20** (2025) 410-416. <http://doi.org/10.5281/zenodo.15739647>
- [16] W. Liu, G. Huo, P. Chen. Clinical benefit of pembrolizumab in treatment of first line non-small cell lung cancer: a systematic review and meta-analysis of clinical characteristics. *BMC Cancer* **23** (2023). <http://doi.org/10.1186/s12885-023-10959-3>
- [17] A. Patnaik, S. Kang, D. Rasco, K. Papadopoulos, J. Elassaiss-Schaap, M. Beeram, A. W. Tolcher. Phase I study of pembrolizumab (MK-3475; Anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clinical Cancer Research* **21** (2015) 4286-4293. <http://doi.org/10.1158/1078-0432.CCR-14-2607>
- [18] L. Pai-Scherf, G. Blumenthal, H. Li, S. Subramaniam, P. Mishra-Kalyani, K. He, R. Pazdur. Approval Summary: Pembrolizumab for Treatment of Metastatic Non-Small Cell Lung Cancer: First-Line Therapy and Beyond. *Oncologist* **22** (2017) 1392-1399. <http://doi.org/10.1634/theoncologist.2017-0078>

- [19] P. Keynote. Pembrolizumab KEYNOTE-001: An adaptive study leading to accelerated approval for two indications and a companion diagnostic. *Annals of Oncology* **28** (2017) 1388-1398. <http://doi.org/10.1093/annonc/mdx076>
- [20] M. Awad, S. Gadgeel, H. Borghaei, A. Patnaik, J. Yang, S. Powell, C. Langer. Long-Term Overall Survival From KEYNOTE-021 Cohort G: Pemetrexed and Carboplatin with or without Pembrolizumab as First-Line Therapy for Advanced Nonsquamous NSCLC. *Journal of Thoracic Oncology* **16** (2021) 162-168. <http://doi.org/10.1016/j.jtho.2020.09.015>
- [21] F. Facchinetti, G. Mazzaschi, F. Barbieri, F. Passiglia, F. Mazzoni, R. Berardi, M. Tiseo. First-line pembrolizumab in advanced non-small cell lung cancer patients with poor performance status. *European Journal of Cancer* **130** (2020) 155-167. <http://doi.org/10.1016/j.ejca.2020.02.023>
- [22] M. Reck. Pembrolizumab as first-line therapy for metastatic non-small-cell lung cancer. *Immunotherapy* **10** (2018) 93-105. <http://doi.org/10.2217/imt-2017-0121>
- [23] V. Alessi, B. Ricciuti, E. Jiménez-Aguilar, F. Hong, Z. Wei, M. Nishino, M. Awad. Outcomes to first-line pembrolizumab in patients PD-L1-high ( $\geq 50\%$ ) non-small cell lung cancer and a poor performance status. *Journal for Immunotherapy of Cancer* **8** (2020) 78-87. <http://doi.org/10.1136/jitc-2020-001007>
- [24] D. Ksienki, E. Wai, D. Alex, N. Croteau, A. Freeman, A. Chan, M. Lesperance. Prognostic significance of the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio for advanced non-small cell lung cancer patients with high PD-L1 tumor expression receiving pembrolizumab. *Translational lung Cancer Research*. **10** (2021) 355-367. <http://doi.org/10.21037/tlcr-20-541>
- [25] S. Ben Dori, A. Aizic, E. Sabo, D. Hershkowitz. Spatial heterogeneity of PD-L1 expression and the risk for misclassification of PD-L1 immunohistochemistry in non-small cell lung cancer. *Lung Cancer* **147** (2020) 91-98. <http://doi.org/10.1016/j.lungcan.2020.07.012>
- [26] Y. Saito, S. Horiuchi, H. Morooka, T. Ibi, N. Takahashi, T. Ikeya, E. Hoshi. Inter-tumor heterogeneity of PD-L1 expression in non-small cell lung cancer. *Journal of Thoracic Disease* **11** (2019) 4982-4991. <http://doi.org/10.21037/jtd.2019.12.24>
- [27] E. Sajjadi, K. Venetis, C. Scatena, N. Fusco. Biomarkers for precision immunotherapy in the metastatic setting: Hope or reality?. *Ecancermedicalscience*, **14** (2020) 78-89. <http://doi.org/10.3332/ECANCER.2020.1150>
- [28] Mhanna, N. Guibert, J. Milia, J. Mazieres. When to Consider Immune Checkpoint Inhibitors in Oncogene-Driven Non-Small Cell Lung Cancer?. *Current Treatment Options in Oncology* **20** (2019) 65-69. <http://doi.org/10.1007/s11864-019-0652-3>
- [29] Stares, T. Ding, C. Stratton, F. Thomson, M. Baxter, H. Cagney, I. Phillips. Biomarkers of systemic inflammation predict survival with first-line immune checkpoint inhibitors in non-small-cell lung cancer. *ESMO Open* **7** (2022) 100445. <http://doi.org/10.1016/j.esmoop.2022.100445>
- [30] K. L. Ayers, M. Ma, G. Debussche, D. Corrigan, J. McCafferty, K. Lee, S. D. Li. A composite biomarker of neutrophil-lymphocyte ratio and hemoglobin level correlates with clinical response to PD-1 and PD-L1 inhibitors in advanced non-small cell lung cancers. *BMC Cancer* **21** (2021) 441. <http://doi.org/10.1186/s12885-021-08194-9>
- [31] Y. Zhou, Z. Lin, X. Zhang, C. Chen, H. Zhao, S. Hong, L. Zhang. First-line treatment for patients with advanced non-small cell lung carcinoma and high PD-L1 expression: Pembrolizumab or pembrolizumab plus chemotherapy. *Journal for Immunotherapy of Cancer* **7** (2019) 120-129. <http://doi.org/10.1186/s40425-019-0600-6>
- [32] E. Fountzilas, S. Lampaki, G. Koliou, A. Koumariou, S. Levva, A. Vagionas, D. Bafaloukos. Real-world safety and efficacy data of immunotherapy in patients with cancer and autoimmune disease: the experience of the Hellenic Cooperative Oncology Group. *Cancer Immunology* **71** (2022) 327-337. <http://doi.org/10.1007/s00262-021-02985-6>
- [33] B. Tomasik, M. Bieńkowski, M. Braun, S. Popat, R. Dziadziuszko. Effectiveness and safety of immunotherapy in NSCLC patients with ECOG PS score  $\geq 2$  - Systematic review and meta-analysis. *Lung Cancer* **158** (2021) 97-106. <http://doi.org/10.1016/j.lungcan.2021.06.004>

- [34] C. J. D. Wallis, M. Butaney, R. Satkunasivam, S. Freedland, S. Patel, O. Hamid, Z. Klaassen. Association of Patient Sex with Efficacy of Immune Checkpoint Inhibitors and Overall Survival in Advanced Cancers: A Systematic Review and Meta-analysis. *JAMA Oncology* **5** (2019) 529-536. <http://doi.org/10.1001/jamaoncol.2018.5904>
- [35] F. Yang, S. Markovic, J. Molina, T. Halfdanarson, L. Pagliaro, A. Chintakuntlawar Y. Wang. Association of Sex, Age, and Eastern Cooperative Oncology Group Performance Status with Survival Benefit of Cancer Immunotherapy in Randomized Clinical Trials: A Systematic Review and Meta-analysis. *JAMA Network Open* **3** (2020) e2012534. <http://doi.org/10.1001/jamanetworkopen.2020.12534>
- [36] Schwartzberg, B. Korytowsky, J. Penrod, Y. Zhang, T. Le, C. Batenchuk, L. Krug. Real-World Clinical Impact of Immune Checkpoint Inhibitors in Patients with Advanced/Metastatic Non-Small Cell Lung Cancer after Platinum Chemotherapy. *Clinical Lung Cancer* **20** (2019) 287-296.e4. <http://doi.org/10.1016/j.clcc.2019.04.004>
- [37] A. F. Kaizal, J. Algburi, M. Al-Haidarey. Heavy metal bioaccumulation in the blood and lungs of white albino rats exposed to welding fume. *Procedia of Environmental Science Engineering and Management* **11** (2024) 83-89. [https://www.procedia-esem.eu/pdf/issues/2024/no1/10\\_Kaizal\\_24.pdf](https://www.procedia-esem.eu/pdf/issues/2024/no1/10_Kaizal_24.pdf)
- [38] K. Amrane. First-line pembrolizumab for non-small cell lung cancer patients with PD-L1  $\geq 50\%$  in a multicenter real-life cohort: The PEMBREIZH study. *Cancer Medicine* **9** (2020) 2309-2316. <http://doi.org/10.1002/cam4.2806>
- [39] G. Wagner, H. K. Stollenwerk, I. Klerings, M. Pecherstorfer, G. Gartlehner, J. Singer. Efficacy and safety of immune checkpoint inhibitors in patients with advanced non-small cell lung cancer (NSCLC): a systematic literature review. *Oncoimmunology* **9** (2020) 45-59. <http://doi.org/10.1080/2162402X.2020.1774314>
- [40] W. You, M. Liu, J. Miao, Y. Liao, Y. Song, D. Cai. A network meta-analysis comparing the efficacy and safety of anti-PD-1 with anti-PD-L1 in non-small cell lung cancer. *Journal of Cancer* **9** (2018) 1200-1206. <http://doi.org/10.7150/jca.22361>
- [41] K. Nesline, T. Knight, S. Colman, K. Patel. Economic Burden of Checkpoint Inhibitor Immunotherapy for the Treatment of Non-Small Cell Lung Cancer in US Clinical Practice. *Clinical Therapeutics* **42** (2020) 1682-1698. <http://doi.org/10.1016/j.clinthera.2020.06.018>
- [42] X. Ma, L. Bellomo, K. Magee, C. Bennette, O. Tymejczyk, M. Samant, A. Bourla Characterization of a Real-World Response Variable and Comparison with RECIST-Based Response Rates from Clinical Trials in Advanced NSCLC. *Advances in Therapy* **38** (2021) 1843-1859. <http://doi.org/10.1007/s12325-021-01659-0>
- [43] L. Paz-Ares, A. Luft, D. Vicente, A. Tafreshi, M. Gümüş, J. Mazières D. Kowalski. Pembrolizumab plus Chemotherapy for Squamous Non-Small-Cell Lung Cancer. *New England Journal of Medicine* **379** (2018) 2040-2051. <http://doi.org/10.1056/nejmoa1810865>
- [44] R. Herbst, P. Baas, D. Kim, E. Felip, J. Pérez-Gracia, J. Han, E. Garon. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *The Lancet* **387** (2016) 1540-1550. [http://doi.org/10.1016/S0140-6736\(15\)01281-7](http://doi.org/10.1016/S0140-6736(15)01281-7)
- [45] C. Langer, S. Gadgeel, H. Borghaei, V. Papadimitrakopoulou, A. Patnaik, S. Powell, L. Gandhi. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *The Lancet Oncology* **17** (2016) 1497-1508. [http://doi.org/10.1016/S1470-2045\(16\)30498-3](http://doi.org/10.1016/S1470-2045(16)30498-3)
- [46] T. Mok, Y. Wu, I. Kudaba, D. Kowalski, B. Cho, B. Turna, G. Martinengo. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *The Lancet* **393** (2019) 1819-1830. [http://doi.org/10.1016/S0140-6736\(18\)32409-7](http://doi.org/10.1016/S0140-6736(18)32409-7)
- [47] S. Ren, J. Feng, S. Ma, H. Chen, Z. Ma, C. Huang, C. Zhou. KEYNOTE-033: Randomized phase 3 study of pembrolizumab vs docetaxel in previously treated, PD-L1-positive, advanced NSCLC. *International Journal of Cancer* **153** (2023) 623-634. <http://doi.org/10.1002/ijc.34532>
- [48] L. Gandhi, D. Rodríguez-Abreu, S. Gadgeel, E. Esteban, E. Felip, M. Garassino. Abstract CT075: KEYNOTE-189: Randomized, double-blind, phase 3 study of pembrolizumab (pembro) or placebo plus

- pemetrexed (pem) and platinum as first-line therapy for metastatic NSCLC. *Cancer Research* **78** (2018) CT075. <https://doi.org/10.1158/1538-7445.AM2018-CT075>
- [49] J. R. Brahmer. Safety profile of pembrolizumab monotherapy based on an aggregate safety evaluation of 8937 patients. *European Journal of Cancer* **199** (2024) 113530. <http://doi.org/10.1016/j.ejca.2024.113530>
- [50] G. Pentheroudakis. Recent eUpdate to the ESMO Clinical Practice Guidelines on early and locally advanced non-small-cell lung cancer (NSCLC). *Annals of Oncology* **31** (2020) 1265-1266. <http://doi.org/10.1016/j.annonc.2020.05.023>
- [51] X. Shen, B. Zhao. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: Meta-analysis. *British Medical Journal* **362** (2018) 43-49. <http://doi.org/10.1136/bmj.k3529>
- [52] F. Passiglia, G. Bronte, V. Bazan, C. Natoli, S. Rizzo, A. Galvano, A. Russo PD-L1 expression as predictive biomarker in patients with NSCLC: A pooled analysis. *Oncotarget* **7** (2016) 19738-19747. <http://doi.org/10.18632/oncotarget.7582>
- [53] R. Ferrara, L. Mezquita, E. Auclin, N. Chaput, B. Besse. Immunosenescence and immunecheckpoint inhibitors in non-small cell lung cancer patients: Does age really matter. *Cancer Treatment Reviews* **60** (2017) 60-68. <http://doi.org/10.1016/j.ctrv.2017.08.003>
- [54] C. Wang. Effect of sex on the efficacy of patients receiving immune checkpoint inhibitors in advanced non-small cell lung cancer. *Cancer Medicine* **8** (2019) 4023-4031. <http://doi.org/10.1002/cam4.2280>
- [55] D. Planchard, S. Popat, K. Kerr, S. Novello, E. Smit, C. Faivre-Finn. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology* **29** (2018) iv192-iv237. <http://doi.org/10.1093/annonc/mdy275>
- [56] J. McLaughlin. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *JAMA Oncology* **2** (2016) 46-54. <http://doi.org/10.1001/jamaoncol.2015.3638>
- [57] C. Tumei, C. Harview, J. Yearley, I. Shintaku, E. Taylor, L. Robert, A. Ribas. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515** (2014) 568-571. <http://doi.org/10.1038/nature13954>
- [58] K. Ninomiya, I. Oze, Y. Kato, T. Kubo, E. Ichihara, K. Rai, K. Hotta. Influence of age on the efficacy of immune checkpoint inhibitors in advanced cancers: a systematic review and meta-analysis. *Acta Oncologica* **59** (2020) 249-256. <http://doi.org/10.1080/0284186X.2019.1695062>
- [59] E. Muchnik, K. Loh, M. Strawderman, A. Magnuson, S. Mohile, V. Estrah, R. Maggiore. Immune Checkpoint Inhibitors in Real-World Treatment of Older Adults with Non-Small Cell Lung Cancer. *Journal of the American Geriatrics Society* **67** (2019) 905-912. <http://doi.org/10.1111/jgs.15750>
- [60] S. L. Klein, K. L. Flanagan. Sex differences in immune responses. *Nature Reviews Immunology* **16** (2016) 626-638. <http://doi.org/10.1038/nri.2016.90>
- [61] K. A. Marrone, W. Ying, J. Naidoo. Immune-Related Adverse Events from Immune Checkpoint Inhibitors. *Clinical Pharmacology & Therapeutics* **100** (2016) 242-251. <http://doi.org/10.1002/cpt.394>
- [62] R. Jin, C. Liu, S. Zheng, X. Wang, X. Feng, H. Li. Molecular heterogeneity of anti-PD-1/PD-L1 immunotherapy efficacy is correlated with tumor immune microenvironment in East Asian patients with non-small cell lung cancer. *Cancer Biology & Medicine* **17** (2020) 768-781. <http://doi.org/10.20892/j.issn.2095-3941.2020.0121>
- [63] C. Zhang, J. Zhang, J. Tan, P. Tian, W. Li. Cost-Effectiveness of Pembrolizumab for the treatment of Non-Small-Cell lung cancer: A systematic review. *Frontiers in Oncology* **12** (2022) 198-205. <http://doi.org/10.3389/fonc.2022.815587>
- [64] F. A. van Delft. Modeling strategies to analyse longitudinal biomarker data: An illustration on predicting immunotherapy non-response in non-small cell lung cancer. *Heliyon* **8** (2022) e10932. <http://doi.org/10.1016/j.heliyon.2022.e10932>
- [65] S. M. Batool, A. Yekula, P. Khanna, T. Hsia, A. Gamblin, E. Ekanayake. The Liquid Biopsy Consortium: Challenges and opportunities for early cancer detection and monitoring. *Cell Reports Medicine* **4** (2023) 101198. <http://doi.org/10.1016/j.xcrm.2023.101198>

- [66] M. Qiao, T. Jiang, S. Ren, C. Zhou. Combination Strategies on the Basis of Immune Checkpoint Inhibitors in Non-Small-Cell Lung Cancer: Where Do We Stand?. *Clinical Lung Cancer* **19** (2018) 1-11. <http://doi.org/10.1016/j.clcc.2017.06.005>
- [67] F. Conforti. Sex-Based Heterogeneity in Response to Lung Cancer Immunotherapy: A Systematic Review and Meta-Analysis. *Journal of the National Cancer Institute* **111** (2019) 772-781. <http://doi.org/10.1093/jnci/djz094>
- [68] V. Velcheti, S. Chandwani, X. Chen, M. Catherine Pietanza, T. Burke. First-line pembrolizumab monotherapy for metastatic PD-L1-positive NSCLC: Real-world analysis of time on treatment. *Immunotherapy* **11** (2019) 889-901. <http://doi.org/10.2217/imt-2019-0061>
- [69] S. Liang, H. Wang, H. Tian, Z. Xu, M. Wu, D. Hua, C. Li. The prognostic biological markers of immunotherapy for non-small cell lung cancer: current landscape and future perspective. *Frontiers in Immunology* **14** (2023) 1249980. <http://doi.org/10.3389/fimmu.2023.1249980>
- [70] A. Prelaj, E. Galli, V. Miskovic, M. Pesenti, G. Viscardi, B. Pedica, A. Pedrocchi. Real-world data to build explainable trustworthy artificial intelligence models for prediction of immunotherapy efficacy in NSCLC patients. *Frontiers in Oncology* **12** (2023) 1078822. <http://doi.org/10.3389/fonc.2022.1078822>