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A comprehensive assessment of treatment outcomes and survival in non-small cell lung cancer patients treated with Pembrolizumab

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A comprehensive assessment of treatment outcomes and survival in non-small cell lung cancer patients treated with Pembrolizumab

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RESUMO

Introdução: O cancro do pulmão é a principal causa de morte por cancro no mundo, apresentando elevadas taxas de mortalidade. Embora seja mais comum em homens, as mortes no sexo feminino estão a aumentar. Com uma taxa de sobrevivência aos 5 anos de apenas 19%, principalmente pelo diagnóstico tardio, este constitui um importante desafio nos cuidados de saúde. Os fatores de risco incluem aspetos não modificáveis como a idade e o sexo, e fatores modificáveis como o tabagismo e exposição ambiental. O diagnóstico atempado, geralmente após sintomas como tosse e dor torácica, e o correto estadiamento são essenciais para um tratamento eficaz. A resseção cirúrgica está indicada nos estadios iniciais, enquanto estadios avançados requerem terapia multimodal, incluindo inibidores de *checkpoints* imunológicos como o Pembrolizumab, que revolucionaram o tratamento do cancro do pulmão de não pequenas células (CPNPC) avançado. Contudo, persistem desafios como a deteção precoce e o desenvolvimento de resistência à terapêutica, enfatizando a necessidade de tratamentos personalizados e da contínua investigação na abordagem do cancro do pulmão.

Objetivos: Este estudo visa avaliar a eficácia do Pembrolizumab no tratamento de doentes com CPNPC em contexto real. Os objetivos principais incluem comparar os resultados do Pembrolizumab em monoterapia versus em combinação com quimioterapia, analisar a eficácia de cada abordagem e comparar os resultados com dados publicados, para estabelecer padrões de referência.

Métodos: Foram analisados retrospetivamente os doentes com CPNPC tratados com Pembrolizumab, em monoterapia ou em combinação, no IPO Porto entre janeiro de 2018 e dezembro de 2022. Parâmetros clinicopatológicos, regimes de tratamento e resultados de sobrevivência, incluindo a sobrevivência global e a sobrevivência livre de progressão, foram recolhidos dos registos clínicos e dos relatórios de Anatomia Patológica. As análises estatísticas foram realizadas utilizando a aplicação *SPSS Statistics*.

Resultados: Um total de 292 doentes foram incluídos no estudo, dos quais 176 receberam Pembrolizumab em monoterapia e 116 Pembrolizumab combinado com quimioterapia como tratamento de primeira linha. As características dos doentes foram descritas, incluindo idade, sexo, *performance status*, histologia, estadio da doença e a expressão tumoral de PD-L1. A mediana da sobrevivência global foi de 19,50 meses para Pembrolizumab em monoterapia e 16,00 meses para Pembrolizumab associado a quimioterapia, enquanto a sobrevivência livre de progressão foi

semelhante entre os grupos (13,00 meses). No entanto, após 12 meses, a sobrevivência livre de progressão foi significativamente maior na coorte de terapia combinada (45% vs. 33%). O estadio da doença influenciou significativamente as sobrevivências da coorte de terapia combinada, conforme confirmado pela análise multivariada.

Conclusões: Este estudo revela a eficácia do Pembrolizumab na gestão do CPNPC, em linha com a literatura internacional, destacando a importância das características dos doentes na previsão dos resultados do tratamento. Estudos prospetivos adicionais são necessários para validar estes achados e otimizar a utilização de Pembrolizumab em diversos contextos clínicos, melhorando os desfechos para os doentes com CPNPC em estadio avançado.

Palavras-chave: Cancro do Pulmão; Cancro do Pulmão de Não Pequenas Células; PD-L1; Imunoterapia; Pembrolizumab; Resultados do Tratamento, Recorrência; Progressão da Doença

ABSTRACT

Background: Lung cancer constitutes the leading cause of cancer-related deaths globally, accounting for significant mortality rates worldwide. Although more commonly diagnosed in men, deaths attributable to lung cancer among women are escalating. With a 5-year survival rate of only 19%, primarily due to detection at late-stages, it poses a critical public health challenge. Risk factors encompass both non-modifiable elements like age and sex, and modifiable factors such as smoking and environmental exposures. Timely diagnosis, often signaled by symptoms like cough and chest pain, and accurate disease staging are essential for effective treatment planning. While surgical resection is indicated for early-stage cases, advanced stages demand multimodal therapy, including immune checkpoint inhibitors like Pembrolizumab, which have transformed treatment for advanced NSCLC by targeting specific pathways. Nevertheless, challenges such as early detection and therapy resistance persist, emphasizing the continuous need for personalized treatments and ongoing research efforts on lung cancer management.

Goals: This study aims to conduct a comprehensive real-world evaluation of the effectiveness of Pembrolizumab in treating non-small cell lung cancer (NSCLC) patients. The primary objectives include assessing treatment outcomes of Pembrolizumab in monotherapy versus in combination with chemotherapy, analyzing their respective efficacies in NSCLC management, and comparing results with published data to establish benchmarks.

Methods: Patients with NSCLC treated with Pembrolizumab, alone or in combination with chemotherapy, at the Portuguese Oncology Institute of Porto between January 2018 and December 2022 were retrospectively analyzed. Clinicopathological parameters, treatment regimens, and survival outcomes including overall survival (OS) and progression-free survival (PFS) were collected from clinical charts and pathology reports. Statistical analyses were conducted using SPSS Statistics software.

Results: A total of 292 patients were enrolled, with 176 receiving Pembrolizumab monotherapy and 116 receiving combination therapy with chemotherapy as first-line treatment. Patient characteristics, including age, sex, performance status, histology, disease stage, and PD-L1 tumor proportion score (TPS) were described. Median OS was 19.50 months for Pembrolizumab in monotherapy and 16.00 months for Pembrolizumab + CT, while PFS was comparable between groups (13.00 months). However, after 12 months, PFS was significantly higher in the combination therapy cohort (45% vs. 33%). Disease stage significantly impacted OS and PFS in the combination

therapy cohort, as confirmed by multivariable analysis.

Conclusions: This study provides insights into Pembrolizumab's efficacy in NSCLC management, in line with published literature. It highlights the importance of patient characteristics in predicting treatment outcomes. Further prospective studies are needed to validate these findings and optimize Pembrolizumab's use in diverse clinical contexts, ultimately improving outcomes for advanced NSCLC patients.

Keywords: Lung Cancer; Non-Small Cell Lung Cancer; PD-L1; Immunotherapy; Pembrolizumab; Treatment Outcomes, Recurrence; Disease progression

LIST OF ABBREVIATIONS

ALK - Anaplastic Lymphoma Kinase

AUC - Area Under the Curve

BRAF - B-Raf Proto-Oncogene Serine/Threonine-Protein Kinase

CI - Confidence Interval

COPD - Chronic Obstructive Pulmonary Disease

CPNPC - Cancro do Pulmão de Não Pequenas Células

CT - Chemotherapy

CTLA4 - Cytotoxic T-Lymphocyte-Associated Protein 4

DGS - Direção Geral da Saúde

DNA - Deoxyribonucleic Acid

EBUS-TBNA - Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration

ECOG PS - Eastern Cooperative Oncology Group Performance Status

EGFR - Epidermal Growth Factor Receptor

ES-SCLC - Extensive Stage Small Cell Lung Cancer

GLOBOCAN - Global Cancer Observatory

HER2 - Human Epidermal Growth Factor

HIV - Human Immunodeficiency Virus

HR - Hazard Ratio

ICIs - Immune Checkpoint Inhibitors

IHC – Immunohistochemistry

KRAS - Kirsten Rat Sarcoma Viral Oncogene

LC – Lung Cancer

LS-SCLC – Limited Stage Small Cell Lung Cancer

MET - Mesenchymal-Epithelial Transition

N.E. - Not Evaluated

N.S. - Not Significant

NAD+ - Nicotinamide Adenine Dinucleotide

NOS - Not Otherwise Specified

NSCLC - Non-Small Cell Lung Cancer

OS - Overall Survival

PCI - Prophylactic Cranial Irradiation

PD-1 - Programmed Cell Death Protein 1

PD-L1- Programmed Death-Ligand 1

PET - Positron Emission Tomography

PFS - Progression-Free Survival

PS – Performance Score

RFA - Radiofrequency Ablation

ROS1 - ROS Proto-Oncogene 1 Kinase

RT - Radiotherapy

SBRT - Stereotactic Body Radiation Therapy

SCC - Squamous Cell Carcinoma

SCLC - Small Cell Lung Cancer

SPSS - Statistical Package for the Social Sciences

TB - Tuberculosis

TME - Tumor Microenvironment

TNM - Tumor, Node, Metastasis

TP53 - Tumor Protein P53

TPS - Tumor Proportion Score

V600E - Val600Glu

VATS - Video-Assisted Thoracoscopic Surgery

WHO - World Health Organization

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INTRODUCTION

Epidemiology

Lung cancer (LC) remains the leading cause of cancer-related death worldwide, claiming an estimated 1.796 million lives annually (18.0% of all cancer deaths).[1,2] Its yearly incidence stands at 2.207 million cases (11.4%), positioning it prominently among the most frequently diagnosed cancers, trailing behind breast cancer, the primary gender-specific malignancy.[1] Incidence rate and clinical manifestations of LC exhibit variations influenced by geographic location, ethnic diversity, tobacco exposure, cigarette types, and other risk factors, as well as environmental elements.[3] Nonetheless, it is particularly incident in regions with high (33.9%) and very high (31.5%) human development index, such as Eastern Asia (45.9%), Northern America (11.5%) and Central and Eastern Europe (6.9%).[1]

Globally, it ranks as the 3rd most common cancer in men (6.4%) and 6th in women (4%), primarily due to disparities in tobacco consumption.[1,2] Since 2012, female mortality has increased 5.1%, while in males there has been a decline of 10.7%, which aligns with the diminishing prevalence of smoking.[3] As per the 2020 GLOBOCAN report, the age-standardized cumulative lifetime risk for the diagnosis of LC is 3.8% in men and 1.8% in women.[1]

Despite remarkable advancements in survival outcomes for various cancers, there have been only slight improvements in the 5-year survival rates, mostly attributed to late-stage diagnoses. Presently, the 5-year relative survival rate for LC stands at 19%.[4]

Regarding Portugal, LC represents the 7th most common cancer (6.3%), and 2020 tendencies reveal an incidence of 11.6% in males and 5.6% in females, claiming the highest mortality rate by cancer (15.9%).[1,5] According to the 2017 report from Direção Geral da Saúde (DGS), over the previous five years male mortality rates experienced a decline of 1.2%, while female mortality increased significantly (16.3%), and future predictions suggest an upward trend in mortality.[3]

Risk Factors

The pathogenesis of LC is the result of the association between exposure to causative agents and individual susceptibility to these agents.[6]

Elements that influence LC risk include lifestyle choices, environmental circumstances, and occupational exposure. The impact of each factor depends on geographic location, sex, racial attributes, genetic predisposition, and the interactions among them. Understanding and addressing this network of influences is crucial for designing personalized and efficient preventive measures and interventions.[7,8]

Non-modifiable risk factors encompass age, sex, race/ethnicity, and family history.

LC is usually diagnosed around age 70, predominantly affecting individuals aged 55-74 (53%), with a notable proportion in those over 75 (37%), while approximately 10% of cases occur in younger adults. Lung tumorigenesis, influenced by factors like telomere shortening and declining NAD+ levels, typically develops decades after initial mutagen exposure.[2]

LC displays a significant gender disparity, with men facing twice the risk due to higher smoking habits, although rates are decreasing in men and rising in women in developed countries. Debate persists on whether women inherently have higher susceptibility to non-small cell lung cancer (NSCLC) despite exhibiting genetic mutations, higher familial risk, and more *ALK* gene rearrangements and *BRAF* mutations in NSCLC. Hormonal factors, including estrogen receptor overexpression and menstrual cycle length, may contribute to the observed differences in LC risk.[2]

African American men have the highest rates of LC, differing among racial/ethnic groups, and incidence is highest among Caucasian women.[2]

Disparities in the manifestation of LC between smokers and non-smokers suggest a genetic influence. A positive family history, particularly among first-degree relatives, increases LC risk, irrespective of smoking history. Therefore, recognizing family history and genetic factors could improve early screening effectiveness.[2,9]

Modifiable risk factors for LC include tobacco consumption, exposure to asbestos, radon, silica or arsenic, air pollution, infections, pre-existing conditions, dietary habits, and physical activity.[6,10,11]

Tobacco smoking is a significant preventable cause of death. Cigarette smoking exposes individuals to nicotine and carcinogens, inducing DNA damage and mutations across organ systems. Exposure to second-hand smoke demonstrates a dose-dependent correlation with LC, impacting non-smokers, pregnant women, and children.[6,10,11] Electronic cigarettes contain potential carcinogens, posing addiction dangers, and heated tobacco products still carry carcinogenic risks.[2,6]

Occupational exposure to carcinogens, primarily asbestos, significantly contributes to LC risk. Inhaling asbestos fibers is linked to various lung pathologies, increasing LC risk. The combination of asbestos exposure and tobacco smoking further intensifies LC risk.[6,12]

Notably, crystalline silica is established as a human carcinogen, therefore workers exposed to this substance encounter an elevated risk of cancer.[13,14]

Radon, a mutagenic gas from uranium decay, poses heightened risk in areas with elevated exposure, notably affecting underground workers. Exposure to residential radon accounts for 10% of cases and can increase to 30% in non-smokers. The combined impact of radon and tobacco smoking intensifies LC risk.[6,15]

Long-term exposure to ambient air pollution is linked to adverse effects such as LC, with carcinogens from fossil fuel combustion and airborne particulate matter posing significant risks.[13,16]

Cities with high particulate matter levels exhibit a 40% increased LC risk, particularly among nonsmokers, and combustion products from wood and charcoal also raise concerns.[6]

Arsenic enters groundwater through leakage of inorganic arsenic, exposing agricultural and industrial workers. Regions with risen arsenic in drinking water show higher LC mortality rates.[6,17]

Infections such as tuberculosis (TB) and human immunodeficiency virus (HIV) are linked to LC. TB increases LC odds by 1.76 times, while HIV independently raises risk by up to 2.5 times. Immunocompromised states also correlate with higher LC rates.[6]

Other comorbidities include Chronic Obstructive Pulmonary Disease (COPD) which independently elevates LC risk, standing as the most prevalent independent risk factor, apart from smoking. Mechanisms contributing to this association involve oxidative stress, damage to DNA, exposure to inflammatory cytokines, suppression of DNA repair mechanisms, and increased cellular proliferation.[6,9]

Consuming a variety of fruits and vegetables, especially those high in antioxidant vitamins (C and E), is suggested to attenuate the susceptibility to LC. Conversely, the ingestion of cured meats, chili, and deep-fried edibles is associated with increased LC risk.[13]

Furthermore, data indicates that individuals who regularly exercise encounter a diminished LC risk and mortality, even among those with heavy smoking history.[13]

Diagnosis

Most LC cases are identified in individuals exhibiting symptoms such as cough, fatigue, dyspnea, chest pain, weight loss, and hemoptysis, the latter holding the highest positive predictive value but being observed in only a fifth of LC cases.[18]

The correlation between early-stage diagnosis and enhanced survival underscores the importance of heightened vigilance from primary care physicians in investigating high-risk patients, even in the absence of symptoms.[18]

The diagnosis (and classification) of LC is crucial for prognosis and the selection of therapeutic interventions.[19,20] Diagnostic procedures for LC involve imaging tests and tissue/pathologic analyses.[18]

Tissue biopsy, often necessary for diagnosing advanced-stage cases, aims to confirm malignancy via histomorphological features, employing immunohistochemistry (IHC) staining for tumor type and grade classification, and providing cellular material for molecular testing geared towards targeted therapy.[19,20]

For centrally located tumors, EBUS–TBNA is a primary procedure, while image-guided transthoracic core needle biopsy may be preferred for peripheral pulmonary lesions.[18]

To mitigate inconclusive or repeated biopsies, a multidisciplinary approach to establish an effective tissue sampling strategy is imperative.[19,20]

Classification

Conventionally, and according to the 2015 WHO classification, lung cancers are categorized into two primary histologic groups: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), the latter account for 85% of LC diagnoses, with adenocarcinoma being the predominant type (40%), while SCLC corresponds to 25-30%, and large cell carcinomas to 10-15%.[2,4,21,22]

As research progressed and a necessity for more meticulous and specific histologic classification occurred, the recently 2021 WHO Classification of Thoracic Tumors categorized lung carcinomas as adenocarcinomas, squamous cell carcinoma (SCC), adenosquamous carcinomas, sarcomatoid carcinomas, large cell carcinomas, neuroendocrine carcinomas (encompassing SCLC), other epithelial tumors and salivary gland-type tumors.[22]

Adenocarcinoma is more prevalent in women and those without a smoking history, typically arising peripherally and exhibiting a mass with central fibrosis and pleural involvement. This epithelial neoplasm demonstrates glandular differentiation and/or mucin production, enabling its classification as adenocarcinoma even in very small biopsy specimens.[4,19]

SCC, the second most prevalent subtype, is histologically characterized by squamous pearls, keratin production, and intercellular bridges. Historically more associated with central lesions, especially in men, there is now an increasing incidence of peripheral tumors.[7]

Adenosquamous carcinoma includes components of both adenocarcinoma and SCC and is often discussed alongside adenocarcinomas due to similar mutation frequencies. Despite these similarities, adenosquamous carcinoma carries a worse prognosis compared to adenocarcinoma and SCC.[19]

Sarcomatoid carcinomas are rare and exhibit poor differentiation, demonstrating diverse pleomorphic, sarcomatoid, and sarcomatous elements. Notably, pleomorphic carcinomas, often large and peripheral, tend to invade the chest wall, indicating a poor prognosis.[23]

Large cell carcinomas are typically situated in the lung periphery, while may also occur centrally and often present as large necrotic masses, which diagnosis requires the exclusion of squamous cell or glandular differentiation.[23]

SCLC, known for its aggressive clinical course, typically manifests as a perihilar mass with early and extensive lymph node metastases. It correlates with smoking history and is often linked to paraneoplastic syndromes.[7]

Despite the relevance of the conventional binary categorization as NSCLC and SCLC, LC may currently be defined by tumor biomarkers and genetic changes, which play a crucial role in tumor

growth and can be targeted through specific interventions or immune checkpoint blockers.[4]

Staging

Cancer staging systems provide a standardized framework to determine the extent of tumor spread, facilitating consistent analysis and communication among diverse sources.[24]

Accurate TNM staging of LC is essential to guide appropriate therapeutic decisions. While surgical resection benefits NSCLC stages I-II, nonsurgical treatments are preferred for more advanced cases. Conventional clinical staging, regularly accomplished with thorax and upper abdomen computed tomography, has limitations in detecting microscopic metastatic disease and distinguishing between malignancy-related and benign reactive hyperplasia-induced enlargement of mediastinal lymph nodes. Positron emission tomography (PET) enhances sensitivity for detecting metabolically active malignant disease, influencing staging and treatment plans in combination with standard work-up. Considering that, guidelines recommend the combined use of computed tomography and PET when possible. For patients at intermediate risk of lymph node metastasis, invasive staging starting with endoscopic ultrasound or endobronchial ultrasound is recommended. Nonetheless, confirmation through mediastinoscopy remains the gold standard for mediastinal staging due to its high specificity and sensitivity.[13,25] Additionally, magnetic resonance imaging of the brain is advised for patients with stage III disease, as the accuracy of clinical staging diminishes in higher stages.[26]

Molecular pathology

LC, characterized by molecular heterogeneity, requires an in-depth comprehension of its biology for effective treatment development. Transitioning from empirical cytotoxic therapy to personalized treatment involves tailoring therapy based on the tumor's genetic profile and PD-L1 status, anticipating benefits from targeted treatments or immune checkpoint inhibitors (ICIs).[27]

The prevalent genetic alterations in lung adenocarcinoma and lung SCC include Kirsten rat sarcoma (*KRAS*) and epidermal growth factor receptor (*EGFR*) mutations. *KRAS* and *EGFR* mutations typically exhibit mutual exclusivity, although coexistence may confer resistance to *EGFR* inhibitors.[27,28]

Tumor protein p53 (*TP53*) mutations increase in frequency with advancing tumor grade, indicating their pivotal role in tumorigenesis. In contrast, *KRAS* mutations in lung adenocarcinoma are consistent across grades, suggesting a more pronounced involvement in tumor initiation or early tumorigenesis.[27,29]

Smokers exhibit an elevated mutation frequency, characterized by cytosine-adenine nucleotide transversions and mutations such as *KRAS* and *TP53*. Conversely, never smokers predominantly

manifest cytosine-thymine transitions, paired with a heightened prevalence of activating *EGFR* mutations, as well as *HER2* mutations and *ROS1* and *ALK* translocations.[4,27,30]

MET exon 14 mutations may also occur in NSCLC (3%-4% of patients) and are particularly common in adenocarcinomas, smokers and older patients, leading to increased *MET* expression. Nonetheless, these mutations are mutually exclusive with other driver mutations including those in *EGFR*, *ALK*, and *BRAF*. Additionally, *BRAF* mutation testing is essential in many countries for advanced non-squamous NSCLC. The most common mutation, V600E, occurs in about 2% of cases, mainly in adenocarcinoma, and is mutually exclusive with *EGFR* and *KRAS* mutations, as well as *ALK* and *ROS1* rearrangements.[31,32]

The tumor microenvironment (TME) undergoes profound changes driven by genetic events, where factors like tumor angiogenesis, immune suppressive/regulatory components, and the cells of the TME play key roles in the tumor progression. In NSCLC, especially among smokers, a high somatic tumor mutation burden may be observed, with metastases displaying more mutations than primary lesions. The heightened clonal neoantigen burden correlates with increased T-cell activity, expressing proteins like PD-L1 and PD-1, suggesting potential sensitivity to ICIs.[27,33]

Treatment

Stage I/II NSCLC

Approximately 25% of individuals diagnosed with NSCLC exhibit stage I or II disease, where achieving a cure is the primary goal.[34]

Surgical resection is the main curative approach, accompanied by lymph node sampling or dissection. Thoracic surgical techniques have improved with video-assisted thoracoscopic surgery (VATS), which is linked to reduced postoperative pain, diminished pulmonary function impairment, and shorter hospital stays. Smokers are encouraged to quit smoking pre-surgery due to higher complication rates and postoperative mortality.[34,35]

Primary radiotherapy (RT) may be considered for those declining surgery or with unresectable tumors, particularly high-risk patients, despite its inferior outcomes compared to surgical resection.[36] Thoracic RT faces a significant challenge due to the risk of normal lung injury. However, technological advancements have enhanced safety and efficacy by enabling precise tumor targeting, minimizing damage to surrounding tissues.[34]

Stereotactic Body Radiation Therapy (SBRT) delivers precise, high-dose radiation to small tumors (<5cm) in few fractions, enhancing tumor control with minimal harm to surrounding tissues. Ideal for medically inoperable stage I NSCLC patients, SBRT does not require a minimum pulmonary function threshold and is associated with limited associated toxicity.[34]

Radiofrequency ablation (RFA) uses high-frequency current via a probe, guided by CT, to induce thermal injury and coagulative necrosis in tumors. Major complications include pneumothorax, pleural effusion, and intrapulmonary hemorrhage. RFA can treat stage I NSCLC in non-surgical candidates or for recurrent tumors.[34]

Distant recurrence is a key cause of mortality within 5 years post-surgery for NSCLC, highlighting the challenge of undetected micrometastases.[34,36]

Stage III NSCLC

Approximately 35% of NSCLC cases are diagnosed at stage III.[34]

The advanced nature of tumors, systemic micrometastases, and heightened distant relapse risk often make primary surgical resection impractical.[34] Resectable stage IIIA NSCLC standard treatment involves surgery followed by chemotherapy, demonstrating significantly prolonged overall survival (OS) rates.[36]

Locally advanced and unresectable stage IIIA benefit from chemotherapy and RT to address local and distant disease.[36] However, concurrent therapy is linked to increased toxicity, particularly esophagitis and pneumonitis.[37]

Adjuvant RT improves local control, but its impact on OS remains uncertain. Nonetheless, for patients with minimal mediastinal lymph node involvement, post-chemotherapy mediastinal RT is reasonable based on retrospective analyses.[34]

Additionally, Durvalumab was approved as a consolidation therapy for stage III disease following either concomitant or sequential chemoradiotherapy.[38]

Stage IV NSCLC

Stage IV NSCLC constitutes 40% of newly diagnosed cases. Treatment depends on comorbidity, performance status, histology, and molecular features; and options include palliative external RT, chemotherapy, combination chemotherapy with targeted therapy, and laser therapy or internal endoscopic RT. Additionally, surgery may be considered to alleviate symptoms.[36]

Currently, the standard treatment for NSCLC without targetable mutations varies depending on PD-L1 expression levels. For patients with PD-L1 TPS <50%, the combination of Pembrolizumab and platinum-based chemotherapy is standard, since it has been shown to significantly improve OS and PFS compared to chemotherapy alone.[39–41] On the other hand, for patients with PD-L1 TPS 50% or higher, Pembrolizumab monotherapy is preferred as first-line treatment, leading to better outcomes in terms of PFS and OS compared to traditional chemotherapy.[42,43] Nonetheless, for stage IV NSCLC patients harboring mutations in *EGFR*, *ALK*, *ROS1*, or *BRAF*, first-line treatment options include approved tyrosine kinase inhibitors, which have transformed therapeutic approaches and

significantly improved patient outcomes. Examples include Erlotinib and Osimertinib for *EGFR* mutations, Alectinib and Brigatinib for *ALK*-positive NSCLC, Crizotinib for patients with *ROS1* rearrangements and the combination of Dabrafenib and Trametinib for those with *BRAF* V600E mutations. These advancements in targeted therapies underscore the importance of genetic profiling in NSCLC, enabling personalized and effective treatment strategies that markedly improve the prognosis for patients with these specific genetic mutations.[44–46]

SCLC

In limited stage small cell lung cancer (LS-SCLC), the primary goal is cure, and combined modality therapy with chemotherapy and radiation is recommended. About 60% of SCLC patients develop brain metastases, and responders are recommended prophylactic cranial irradiation (PCI) to reduce brain metastases incidence.[34]

For extensive stage SCLC (ES-SCLC), standard treatment involves platinum-based chemotherapy, along with PCI for responders. Nonetheless, the addition of Atezolizumab to Carboplatin and Etoposide is also approved as first-line treatment for ES-SCLC, endowing significantly longer OS and PFS than chemotherapy alone.[47] Recurrent disease may be addressed with monotherapy chemotherapy, palliative radiotherapy, or enrollment in clinical trials.[34]

Recurrence is common in ES-SCLC and limited stage small cell lung cancer (LS-SCLC), categorized as resistant or sensitive. Prolonged initial response may favor reinitiating initial chemotherapy, while those relapsing sooner may benefit from second-line agents. [34]

Single-agent chemotherapy is preferred for recurrent SCLC with good performance status due to excessive toxicity and no improvement in OS with combination regimens.[34]

PD-L1 targeted immunotherapy

In the past decade, the treatment approach for advanced NSCLC has transformed significantly. The identification of oncogenes introduced effective treatment targets, notably increasing survival for patients with corresponding driver mutations. Immune-based therapies, particularly inhibitors of the programmed cell death protein 1–programmed death ligand 1 (PD-1/PD-L1) immune checkpoint, have recently improved outcomes in NSCLC treatment, extending survival for individuals with locally advanced disease.[48–50]

Recent publications have assessed PD-1 and PD-L1 expression in NSCLC. PD-1 expression was more common in male smokers with adenocarcinoma, whereas PD-L1 was more prevalent in female non-smokers with adenocarcinoma.[51–53]

The PD-1 receptor, located in cytotoxic T-cells and T-regulatory cells, activates during inflammation or infection. PD-L1 binds to PD-1, deactivating T-cells and suppressing the immune

response, aiding immune evasion by cancer cells. Anti-PD-1 or anti-PD-L1 therapies disrupt this process, enabling activated cytotoxic T-cells to pinpoint and assail cancer cells.[54]

In the absence of targetable genetic alterations and contraindications to PD-1/PD-L1 inhibitors, immunotherapy has emerged as the standard of care for advanced squamous and non-squamous LC. Key ICIs utilized in advanced NSCLC include anti-PD-1 agents (Pembrolizumab/Nivolumab), anti-PD-L1 inhibitor (Atezolizumab), and anti-CTLA4 inhibitor (Ipilimumab).[49]

Pembrolizumab, a revolutionary anti-PD-1 ICI, is a humanized monoclonal antibody. By blocking the PD-1 receptor on T-cells, it activates tumor-specific immune responses, rendering cancer cells vulnerable to destruction. This therapy has shown significant efficacy in various advanced malignancies, including NSCLC, melanoma, head and neck SCC, and urothelial carcinoma.[55]

Single-agent ICIs offer a significant advantage in NSCLC, particularly in patients with high PD-L1 expression (TPS ≥50%), observed in up to 30% of cases.[33,48] This can be observed in the phase III trial KEYNOTE-024, where Pembrolizumab demonstrated efficacy in untreated stage IV NSCLC compared to platinum chemotherapy. Among those with PD-L1 TPS ≥50%, Pembrolizumab monotherapy demonstrated a superior response rate and OS.[49,56,57] Oppositely, when PD-L1 expression is <50%, the benefit of using PD-1 or PD-L1 inhibitors as a single agent in the first-line setting is limited compared to platinum-based chemotherapy.[33]

Furthermore, Pembrolizumab (Keytruda™) was assessed in stage IIIB or stage IV NSCLC patients, showing an overall response rate of 19.4%, with higher rates in previously untreated patients.[54,58] Additionally, the efficacy of combining Pembrolizumab and chemotherapy (CT) was investigated in squamous NSCLC patients, demonstrating a 5-year OS rate of 18.4% with Pembrolizumab + CT versus 9.7% with placebo + CT, as well as a 5-year PFS rate of 10.8% versus 3.5% (Pembrolizumab + CT and placebo + CT, respectively), suggesting that combining Pembrolizumab with chemotherapy could be more effective for treating squamous NSCLC, as seen in the KEYNOTE-407 trial.[59] Similarly, the KEYNOTE-189 trial, which investigated Pembrolizumab combined with pemetrexed and platinum chemotherapy in non-squamous NSCLC, reported a 5-year OS rate of 19.4% and 11.3% for patients who received Pembrolizumab plus platinum-Pemetrexed and placebo plus platinum-Pemetrexed, respectively, as well as a 5-year PFS rate of 7.5% versus 0.6%. These trials underscore the transformative impact of Pembrolizumab added to CT in enhancing survival and response rates in both squamous and non-squamous NSCLC.[60]

Nonetheless, it is worth noting that PD-L1 expression as a standalone biomarker may not comprehensively predict treatment outcomes. Despite notable results, patients undergoing ICI therapy may experience disease progression due to acquired resistance. Moreover, a significant portion of patients with high PD-L1 expression may not respond to ICI therapy.[33,61]

OBJECTIVES

The overarching goal of this project is to conduct a comprehensive real-world evaluation of the therapeutic efficacy of immunotherapy, specifically regarding Pembrolizumab in the management of NSCLC patients. The ultimate objective is to help optimize the existing treatment modalities and contribute to establishing new therapeutic standards. The primary objectives of this research project are as follows:

- Conduct an in-depth assessment of treatment outcomes in patients undergoing monotherapy with Pembrolizumab and those undergoing combination therapy with Pembrolizumab and chemotherapy.
- Conduct a comparative analysis of both therapeutic approaches and their respective efficacies in managing the disease and enhancing overall patient survival in NSCLC, stratified according to standard clinical and pathological parameters.
- Compare the results of a single reference institution (IPO Porto) with published data, allowing for benchmarking of clinical results and outcomes.

METHODS

Patient cohorts and clinicopathological parameters

Patients diagnosed with NSCLC, meeting the specified criteria, which were diagnosed, clinically evaluated, and treated with Pembrolizumab at Portuguese Oncology Institute of Porto (IPO Porto) within the time span of 01/2018 to 12/2022 were enrolled in this study.

After identification of patients eligible for this retrospective study, two databases were constructed (one for patients treated with Pembrolizumab in monotherapy and another for patients treated with Pembrolizumab in combination with CT, both as first line) and relevant clinical data, including response to therapy and survival outcome measures, were retrieved from the clinical charts. Clinical data comprised dates of birth, diagnosis, treatment start, recurrence, death, clinical stage, treatments performed, last follow-up date and vital status. Follow-up was updated as of April 30th, 2024. OS was measured from the date of histopathological diagnosis to the date of demise or of last follow-up, whereas PFS was calculated from the date of diagnosis to the date of imagiology or pathology confirmed relapse.

Pathology data, including tumor type and PD-L1 expression (clone 22C3, Dako), assessed according to established guidelines for TPS, were collected from pathology reports.

This study was approved by the Ethics Committee (Comissão de Ética para a Saúde) of IPO Porto (CES.108/024).

Treatment regimens

For patients treated with Pembrolizumab in monotherapy, the regimen consisted of Pembrolizumab 200mg, intravenously, every 3 weeks for 35 cycles.

As for patients treated with Pembrolizumab in combination with CT, the standard regimen comprised Pembrolizumab 200mg, with Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 or Carboplatin AUC 5mg/ml/min intravenously every 3 weeks for 4 cycles, followed by Pembrolizumab 200mg and Pemetrexed 500mg/m^2 intravenously at every 3 weeks for 31 cycles for non-squamous NSCLC. Additionally, for SCC it consisted of intravenous Pembrolizumab 200mg and Carboplatin AUC 6mg/ml/min on Day 1 of each 21-day cycle for 4 cycles, and Paclitaxel 200 mg/m2 on Day 1 of each 21-day cycle for 4 cycles, followed by Pembrolizumab 200mg at every 3 weeks for 31 cycles.

Statistical analysis

For organization of all data, clinical and pathological information retrieved from the electronic patient records were entered into Microsoft Excel files, protected with password, allowing for

subsequent analysis.

Continuous variable distribution between groups was compared utilizing nonparametric tests (Mann-Whitney or Kruskall-Wallis, as appropriate). Kaplan-Meier nonparametric estimator was employed for constructing survival curves. Survival between groups was compared using log-rank test. Hazard ratios (HR) and respective 95% Confidence Intervals (CI) were estimated using Cox-regression models. Statistical significance was set at $p<0.05$. Statistical analysis was performed using SPSS Statistics for Windows, version 27.0 (SPSS, Chicago, IL).

RESULTS

Cohorts' Characterization

A total of 292 patients were enrolled in this study and the respective clinicopathological features are disclosed in **Table I**. Of these, 176 patients received Pembrolizumab monotherapy (Cohort #1) and 116 received Pembrolizumab in combination with chemotherapy (Cohort #2).

Median age at diagnosis was 68 years (range 43-87 years) and 64 years (range 34-81 years) in Cohort #1 and Cohort #2, respectively. In both cohorts most patients were male, representing 133 (75.6%) in Cohort #1 and 85 (73.3%) in Cohort #2.

Among the 176 patients of Cohort #1, n=60 (34.1%) had an ECOG PS of 0, n=108 (61.4%) had a PS of 1, n=6 (3.4%) had a PS of 2, and n=2 (1.1%) had a PS of 3. Additionally, among the 116 patients in Cohort #2, n=37 (31.9%) had an ECOG PS of 0, n=78 (67.2%) had a PS of 1, and n=1 (0.9%) had a PS of 2.

Regarding histology, the majority of tumors in both cohorts were adenocarcinomas, with 111 (63.4%) in Cohort #1 and 83 (71.6%) in Cohort #2, followed by SCC (n=45 (25.7%) and n=24 (20.7%) in Cohort #1 and Cohort #2, respectively) and NSCLC not otherwise specified (n=15 (8.6%) and n=7 (6.0%) in Cohort #1 and Cohort #2, respectively). Additionally, n=4 patients (2.3%) in Cohort #1 and n=2 patients (1.7%) in Cohort #2 presented with other tumor types, specifically adenosquamous, mixed, and neuroendocrine carcinomas.

Most tumors were diagnosed at an advanced stage, comprising 150 (85.2%) and 100 (87.0%) patients in Cohort #1 and Cohort #2, respectively, presenting with stage IV disease, while the remaining patients exhibited stage III disease at diagnosis (n=26 (14.8%) and n=15 (13.0%) in Cohort #1 and Cohort #2, respectively).

A comparison of patient characteristics between both cohorts revealed that the number of patients with high PD-L1 expression (TPS ≥50%) was notably higher in Cohort #1 than in Cohort #2, as expected (n=176 (100%) and n=2 (1.8%) in Cohort #1 and Cohort #2, respectively). Therefore, all patients treated with Pembrolizumab in monotherapy (Cohort #1) exhibited high PD-L1 expression (TPS ≥50%). However, patients treated with Pembrolizumab + CT (Cohort #2) showed a more balanced distribution across various levels of PD-L1 expression, with n=34 (30.1%) at TPS <1%, n=26 (23.0%) at TPS 1-5%, n=23 (20.4%) at TPS 5-10%, n=20 (17.7%) at TPS 10-25%, n=8 (7.1%) at TPS 25-49%, and n=2 (1.8%) at TPS ≥50%.

Outcome Analysis

At the time of data cutoff, 121 (68.8%) and 63 deaths (54.3%) had occurred in Cohort #1 and Cohort #2, respectively (**Table II**). Median (95% CI) OS was 19.50 months (IQR 8.00-36.75 months) in Cohort #1 and 16.00 months (IQR 10-28 months) in Cohort #2. OS was not significantly different between the two cohorts (HR 0.864; 95% CI, 0.635-1.176; p=0.353). - **Figure 1**

Furthermore, 145 (82.4%) patients in Cohort #1 and 62 (53.4%) patients in Cohort #2 experienced disease progression (**Table II**). Median (95% CI) PFS was 13.00 months (IQR: 4.00-26.00 months) in Cohort #1 and 13.00 months (IQR: 6.00-26.00 months) in Cohort #2. Although the median PFS was the same in both cohorts, after 12 months of follow-up there is a clearly visible difference in the behavior of survival curves, which translates into 33% PFS in Cohort #1 and 45% PFS in Cohort #2 and this difference is statistically significant (HR 1.586; 95% CI, 1.176-2.139; p=0.003). - **Figure 2**

Overall Survival Analysis

Univariable Analysis

In Cohort #1, neither sex (**Figure 3**), stage at diagnosis (**Figure 4**) nor ECOG PS demonstrated significant effects on OS. However, age (as continuous variable) (HR 1.021, 95% CI 1.0003-1.042; p=0.046) and histology (p=0.048) (**Figure 5**) significantly impacted OS, particularly in the "other" category compared to adenocarcinomas (HR 3.930, 95% CI 1.411-10.947; p=0.009), while neither SCC nor NSCLC NOS demonstrated a significant impact on OS (p>0.05). - **Table III**

Alternatively, in Cohort #2, the only factors that significantly impacted OS were disease stage (HR 6.310, 95% CI 1.953-20.383; p<0.001) (**Figure 6**) and ECOG PS (p=0.028), while sex (**Figure 7**), age, histology (**Figure 8**) and PD-L1 TPS did not disclose a significant influence on OS (p>0.05). Consequently, an ECOG PS of 1 (HR 2.312, 95% CI 1.250-4.277; p=0.008) showed a significant impact on OS, contrary to an ECOG PS of 2 (p>0.05). - **Table IV**

Multivariable analysis

Considering Cohort #1, both age and histology lost their significance (p>0.05) in multivariable analysis, meaning that they do not hold independent prognostic value. - **Table III**

Furthermore, in Cohort #2, ECOG PS also lost its significance, contrasting with disease stage, which retained an important impact on OS (HR 9.056, 95% CI 2.629-31.197; p<0.001). - **Table IV**

Progression-Free Survival Analysis

Univariable analysis

Regarding the Pembrolizumab monotherapy cohort, only histology (p=0.036) exhibited a significant impact on PFS (**Figure 9**). Other variables, including sex (**Figure 10**), stage at diagnosis (**Figure 11**), age and ECOG PS did not demonstrate significant effects on PFS. Therefore, both SCC and NSCLC NOS did not impact PFS (p>0.05), whereas adenosquamous, mixed, and neuroendocrine carcinomas demonstrated a significant influence on PFS (HR 4.279, 95% CI 1.522-12.033; p=0.006). - **Table III**

On the other hand, in Cohort #2 only disease stage at diagnosis (**Figure 12**) disclosed a significant impact on PFS (HR 2.407, 95% CI 1.078-5.374; p=0.010), whereas sex (**Figure 13**), age, ECOG PS, histology (**Figure 14**), and PD-L1 TPS did not significantly affect PFS. - **Table IV**

Multivariable analysis

Multivariable analysis was not conducted in Cohort #1 nor in Cohort #2 because only histology and disease stage, respectively, significantly associated with survival. - **Table III** and **Table IV**

DISCUSSION AND CONCLUSIONS

LC is the leading cause of cancer-related deaths globally, affecting predominantly men and depicting a poor 5-year survival rate. Treatment is often challenging and encompasses various strategies, including immune checkpoint inhibitors such as Pembrolizumab, which show effectiveness for advanced NSCLC. This study sought to evaluate Pembrolizumab's efficacy in the treatment of NSCLC through a real-world assessment. The primary goal was, then, to assess how the results in a single reference center – IPO Porto – compared to those in the scientific literature. The study was conducted retrospectively and thoroughly examined treatment outcomes in patients receiving Pembrolizumab in monotherapy or in combination with chemotherapy. A comparative analysis of both strategies was performed to determine their efficacy in disease management and patient survival improvement.

The study cohorts' demographic and clinical characteristics revealed a predominance of male patients, with a median age at diagnosis of 68 years old for the Pembrolizumab in monotherapy cohort and 64 years for the Pembrolizumab + CT combination cohort. The majority of patients presented with advanced stage IV disease, which aligns with the typically late diagnosis of NSCLC. The distribution of histological subtypes, mostly adenocarcinomas, is consistent with the typical epidemiology of NSCLC. Thus, the two cohorts are globally representative of LC patient populations routinely diagnosed and treated at tertiary care centers.[62–64]

The outcome analysis demonstrates that OS and PFS outcomes were comparable between the two cohorts. Pembrolizumab in monotherapy prolonged median OS by 3.50 months (19.50 months vs. 16.00 months) when compared to Pembrolizumab in combination with chemotherapy, but this difference did not reach statistical significance. This finding aligns closely with results from a multicenter retrospective trial [65], which reported similar OS outcomes in a real-world setting, with no statistically significant differences observed in the OS, emphasizing the importance of selecting patients based on PD-L1 expression to optimize treatment efficacy. On the other hand, the KEYNOTE-024[42] trial demonstrated a significant survival advantage for Pembrolizumab monotherapy over platinum-based chemotherapy in patients with PD-L1 TPS ≥50%, showing a median OS of 30.0 months compared to 14.2 months for chemotherapy, therefore underscoring the efficacy of Pembrolizumab monotherapy in tumors with high PD-L1 expression, a finding mirrored in our study in which all patients under monotherapy disclosed high PD-L1 expression levels (TPS ≥50%).

While this study indicated no significant difference in median PFS between the two cohorts (13.00 months for both), a significant difference emerged after 12 months, with Pembrolizumab in combination with chemotherapy endowing a significantly improved PFS to patients with PD-L1 TPS

<50% compared to those of Cohort #1. This delayed benefit is consistent with findings from previous trials[40,56,66], which reported enhanced PFS with Pembrolizumab plus chemotherapy in both non-squamous and squamous NSCLC. These studies highlighted the particularly pronounced benefit of combination therapy in patients with low or negative PD-L1 expression, aligning with our observation of improved PFS in the diverse PD-L1 expression group treated with Pembrolizumab plus chemotherapy.

Univariable analysis revealed that although the disease stage at diagnosis was not a significant predictive factor of both OS and PFS in Cohort #1, it was a significant determinant in Cohort #2, influencing both OS and PFS. Indeed, patients with stage IV disease had notably worse outcomes than those detected at earlier stages in Cohort #2. Interestingly, in the KEYNOTE-042[67] trial, although Pembrolizumab in monotherapy improved OS across all PD-L1 expression levels, it was most beneficial for patients with high PD-L1 expression and earlier disease stages. Similarly, real-world studies from European[68] and French[69] cohorts corroborate the critical impact of disease stage and performance status on survival, emphasizing the necessity of early diagnosis and tailored treatment approaches. In Cohort #1, age and histology impacted OS, although these factors lost significance in multivariable analysis, suggesting the absence of independent prognostic significance. This aligns with broader findings in the literature[57,65,70], where histological subtypes like adenocarcinoma are common, but their impact on prognosis is often overshadowed by other clinical factors such as disease stage, PD-L1 expression levels, and ECOG performance status. Furthermore, in Cohort #2, ECOG PS significantly affected OS, with patients with an ECOG PS of 1 showing poorer outcomes than those with an ECOG PS of 0, suggesting that patient functional status plays a critical role in determining the benefit of combination therapy. This finding echoed in studies such as KEYNOTE-407[40], KEYNOTE-024[42] and a multicenter retrospective trial[65], in which patient functional status, as measured by ECOG performance status, emerged as a key determinant of treatment outcomes.

In addition to the clinical criteria included in this study, several other variables may significantly influence survival outcomes in patients with advanced NSCLC. Genetic and molecular characteristics, such as the presence of specific mutations (e.g., in *EGFR*, *ALK*, *KRAS*) and tumor mutational burden, play crucial roles in determining responsiveness to targeted therapies and immunotherapies.[71] Environmental factors, including smoking history[11,72] and exposure to pollutants[73], also impact disease progression and treatment efficacy. Socioeconomic status and access to healthcare resources can lead to disparities in diagnosis, treatment initiation, and adherence, further affecting survival rates.[74,75] Additionally, comorbidities and overall health status, often quantified through performance status scales like ECOG, influence both treatment decisions and patient tolerance to therapy side effects. Recognizing these multifaceted influences is essential

for developing comprehensive, individualized treatment strategies that enhance survival and quality of life for patients with advanced NSCLC.

While this study provides important real-world evidence, there are some limitations that might have impacted the overall analysis, specifically the fact that it was a retrospective study, which introduces inherent limitations that include potential selection bias, variability in data quality, and lack of randomization, factors that can affect the generalizability of the findings. Moreover, the two cohorts were substantially different regarding the distribution of PD-L1 expression, as expected, considering the indications for therapy with Pembrolizumab. All patients in the monotherapy group had high PD-L1 expression (TPS ≥50%), which is consistent with the use of Pembrolizumab in monotherapy. In contrast, the combination therapy group exhibited a more varied distribution of PD-L1 expression levels, although no significant association was disclosed between those levels and patient outcome. Considering that OS was comparable between the two patient cohorts, it might be concluded that the two therapeutic strategies have similar effectiveness when applied to the appropriate setting. Furthermore, a small subset of patients had to be excluded from some variables due to incomplete clinical data. Despite efforts to gather comprehensive information, and regarding the retrospective nature of this study, certain patients lacked essential clinical variables necessary for thorough analysis. Consequently, to uphold the integrity and accuracy of the study, this subset was omitted from certain variables in the final dataset. While this limitation may impact the generalizability of the findings to the broader patient population, steps were taken to ensure the remaining dataset's robustness and representativeness.

In conclusion, this study provides valuable insights into the comparative efficacy and prognostic determinants of Pembrolizumab in monotherapy versus in combination with CT in advanced NSCLC. Additionally, it underscores the importance of patient characteristics, such as disease stage, histology, age, and performance status, in predicting treatment outcomes. Furthermore, while confirming the efficacy of Pembrolizumab in monotherapy in managing advanced NSCLC among patients with high PD-L1 expression, the study suggests that the addition of chemotherapy may provide better disease management and extended PFS for patients with NSCLC disclosing lower PD-L1 expression, which are deemed to be less responsive to Pembrolizumab alone. Recognizing prognostic markers such as disease stage and histological subtype underscores the importance of individualized treatment strategies tailored to patient-specific clinical and pathological characteristics. An interesting question is whether the addition of chemotherapy to Pembrolizumab in patients carrying NSCLC with PD-L1 TPS ≥50% might further improve OS and PFS. Thus, future prospective studies are needed to validate this hypothesis and further elucidate on the optimal use of Pembrolizumab across diverse clinical scenarios. Such efforts will contribute to refining treatment approaches and improving outcomes for patients with advanced NSCLC.

APPENDIX

Tables

Table I *- Demographic and Disease Characteristics of the Patients at Baseline.*

Characteristic	Pembrolizumab (Monotherapy)	Pembrolizumab + Chemotherapy (Combination Therapy)
Number of patients	176	116
Age — yr		
Median	68.0	64.00
Range	43-87	34-81
Sex — no. (%)		
Male	133 (75.6)	85 (73.3)
Female	43 (24.4)	31 (26.7)
ECOG PS — no. (%)		
0	60 (34.1)	37 (31.9)
1	108 (61.4)	78 (67.2)
2	6 (3.4)	1 (0.9)
3	2 (1.1)	0
Histology — no. (%)		
Adenocarcinoma	111 (63.4)	83 (71.6)
Squamous Cell Carcinoma	45 (25.7)	24 (20.7)
Non-Small Cell Lung Cancer	15 (8.6)	7 (6.0)
Others	4 (2.3)	2 (1.7)
Stage — no. (%)		
III	26 (14.8)	15 (13.0)
IV	150 (85.2)	100 (87.0)
PD-L1 TPS — no. (%)		
<1%	0	34 (30.1)
1-5%	0	26 (23.0)
5-10%	0	23 (20.4)
10-25%	0	20 (17.7)
25–49%	0	8 (7.1)
≧50%	176 (100%)	2 (1.8)

Table II *- Outcome analysis of overall survival and progression-free survival in both cohorts (Pembrolizumab in monotherapy and in combination with chemotherapy).*

Overall Survival			
	Pembrolizumab (Monotherapy)	**Pembrolizumab + Chemotherapy (Combination Therapy)**	**Total**
Alive	55 (31.3%)	53 (45.7%)	108 (37.0%)
Dead	121 (68.8%)	63 (54.3%)	184 (63.0%)
Total	176	116	292
Progression-Free Survival			
	Pembrolizumab (Monotherapy)	**Pembrolizumab + Chemotherapy (Combination Therapy)**	**Total**
Not progressed	31 (17.6%)	54 (46.6%)	85 (29.1%)
Progressed	145 (82.4%)	62 (53.4%)	207 (70.9%)
Total	176	116	292

Table III - *Survival analysis in the cohort of patients treated with Pembrolizumab in monotherapy.*

Overall Survival		
	Univariable	**Multivariable***
Sex	N.S.	-
Stage	N.S.	-
Age	p=0.046; HR 1.021, 95% CI 1.0003-1.042	N.S.
ECOG PS	N.S.	-
Histology	p=0.048 SCC vs. Adenocarcinoma: N.S. NSCLC NOS vs. Adenocarcinoma: N.S. Others vs. Adenocarcinoma: p=0.009; HR 3.930, 95% CI 1.411-10.947	N.S.
Progression-Free Survival		
	Univariable	**Multivariable***
Sex	N.S.	-
Stage	N.S.	-
Age	N.S.	-
ECOG PS	N.S.	-
Histology	p=0.036 SCC vs. Adenocarcinoma: N.S. NSCLC NOS vs. Adenocarcinoma: N.S. Others vs. Adenocarcinoma: p=0.006; HR 4.279, 95% CI 1.522-12.033	N.E.

Notes: **ECOG PS** - Eastern Cooperative Oncology Group Performance Status; **N.E.** – Not Evaluated; **N.S.** – Not Significant (p>0.05); **NSCLC NOS** - Non–Small-Cell Lung Cancer-Not Otherwise Specified; **SCC** – Squamous Cell Carcinoma; ***** - adjusted HR

Table IV - *Survival analysis in the cohort of patients treated with Pembrolizumab + chemotherapy (Combination Therapy)*

Overall Survival		
	Univariable	**Multivariable***
Sex	N.S.	-
Stage	p<0.001 HR 6.310, 95% CI 1.953-20.383	p<0.001 HR 9.056, 95% CI 2.629-31.197
Age	N.S.	-
ECOG PS	p=0.028 1 vs. 0: p=0.008; HR 2.312, 95% CI 1.250-4.277 2 vs. 0: N.S.	N.S.
Histology	N.S.	-
PD-L1 TPS	N.S.	-
Progression-Free Survival		
	Univariable	**Multivariable***
Sex	N.S.	-
Stage	p=0.010 HR 2.407, 95% CI 1.078-5.374	N.E.
Age	N.S.	-
ECOG PS	N.S.	-
Histology	N.S.	-
PD-L1 TPS	N.S.	-

Notes: **ECOG PS** - Eastern Cooperative Oncology Group Performance Status; **N.E.** – Not Evaluated; **N.S.** – Not Significant (p>0.05); **NSCLC NOS** - Non–Small-Cell Lung Cancer-Not Otherwise Specified; **PD-L1 TPS** - Programmed Death-Ligand 1 Tumor Proportion Score (TPS); **SCC** – Squamous Cell Carcinoma; ***** - adjusted HR

Figures

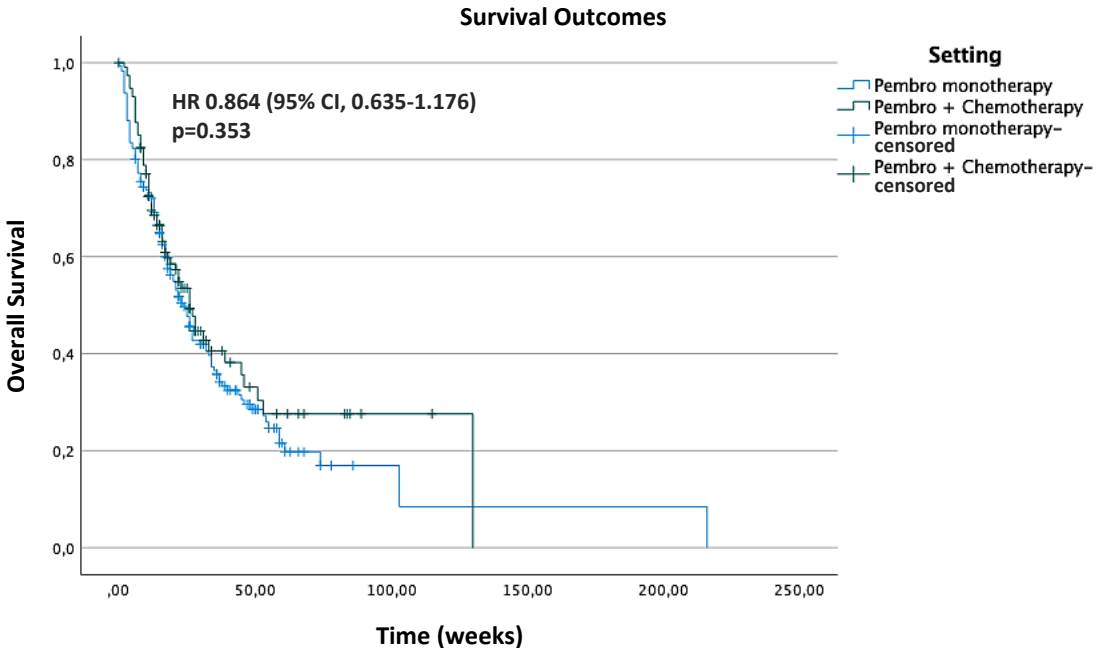

Figure 1 *- Kaplan–Meier analysis of overall survival in both cohorts (Pembrolizumab in monotherapy and in combination with chemotherapy).*

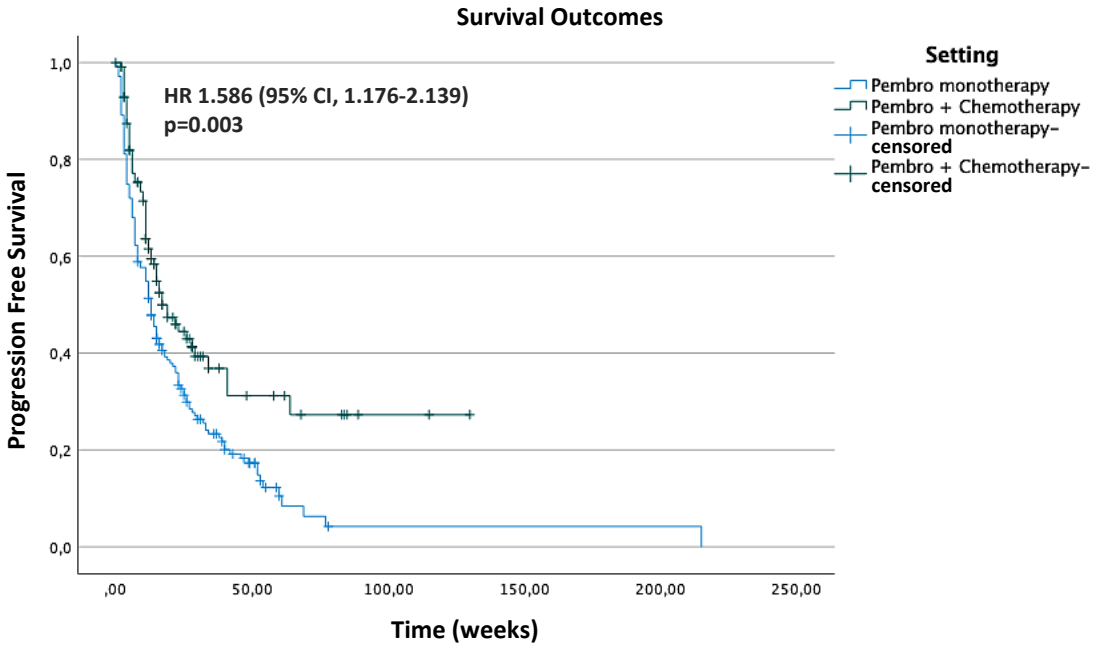

Figure 2 *- Kaplan–Meier analysis of progression-free survival in both cohorts (Pembrolizumab in monotherapy and in combination with chemotherapy).*

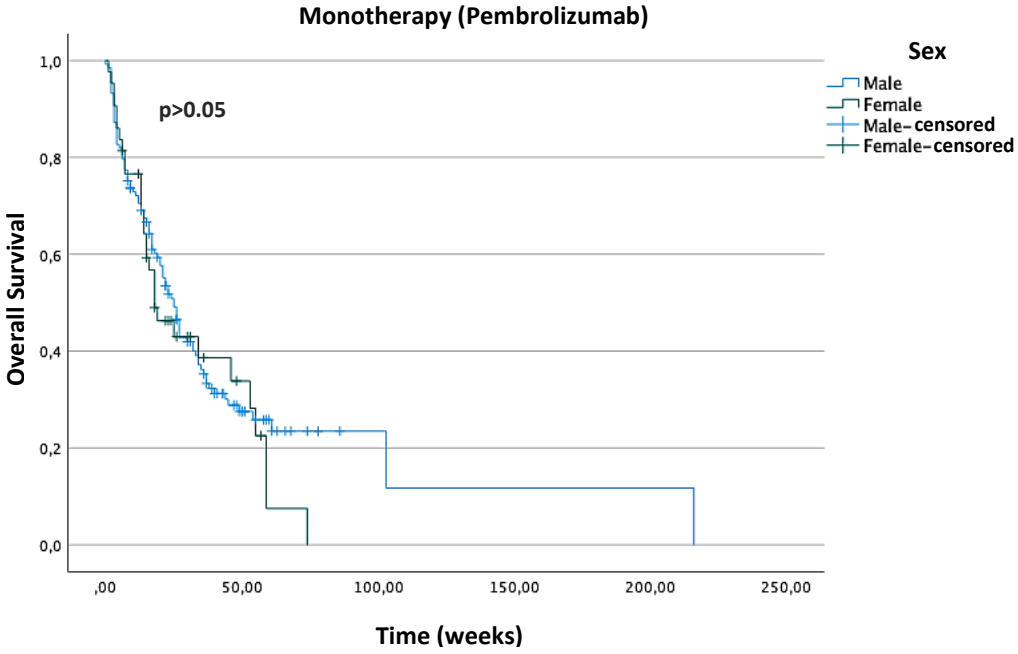

Figure 3 *- Kaplan–Meier analysis of overall survival in patients treated with Pembrolizumab in monotherapy (Cohort #1) stratified by sex.*

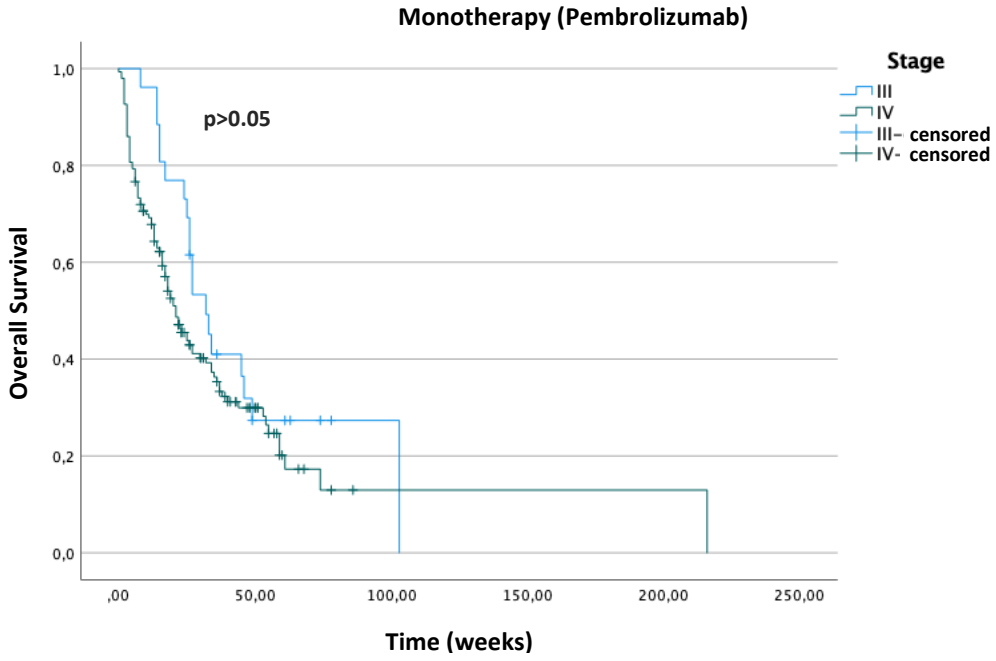

Figure 4 *- Kaplan–Meier analysis of overall survival in patients treated with Pembrolizumab in monotherapy (Cohort #1) stratified by stage.*

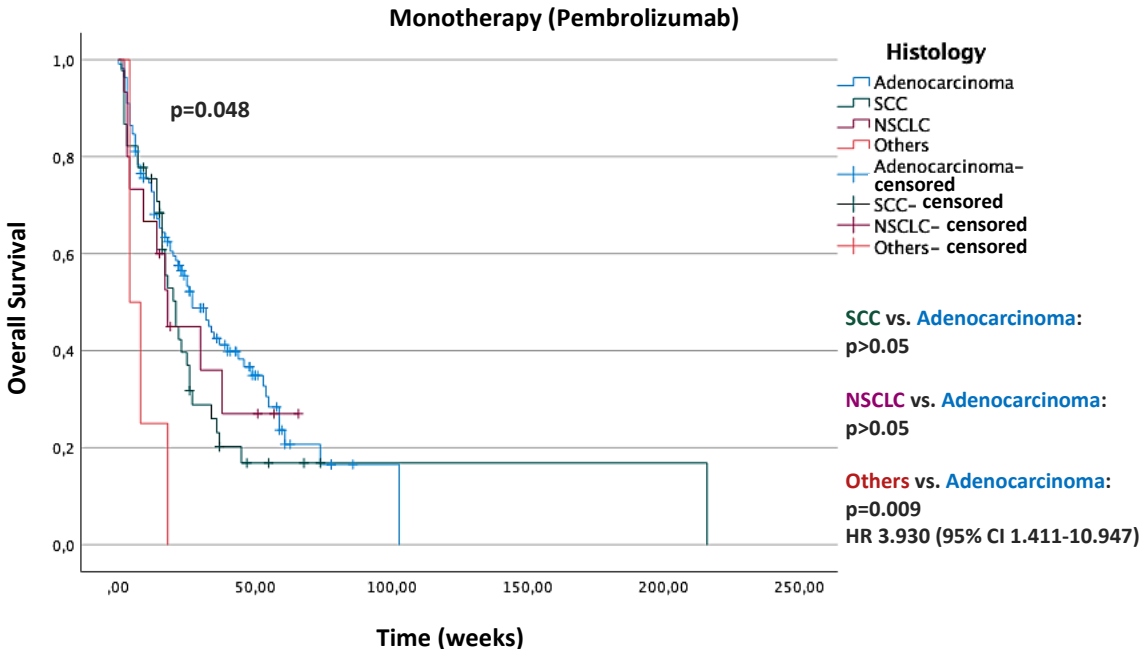

Figure 5 *-Kaplan–Meier analysis of overall survival in patients treated with Pembrolizumab in monotherapy (Cohort #1) stratified by histology.*

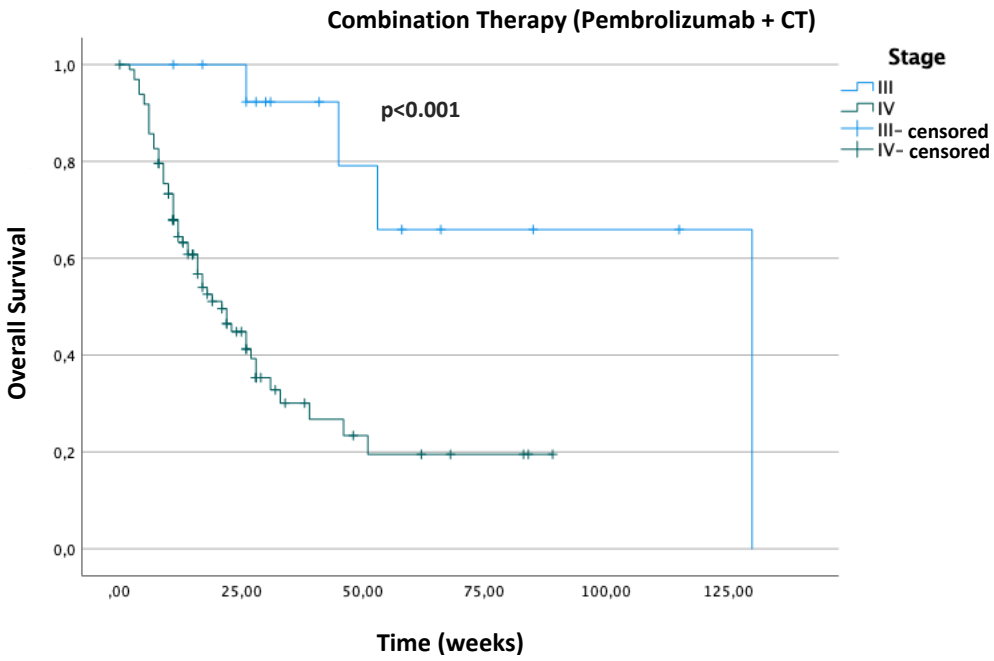

Figure 6 *- Kaplan–Meier analysis of overall survival in patients treated with Pembrolizumab + CT in combination therapy (Cohort #2) stratified by stage.*

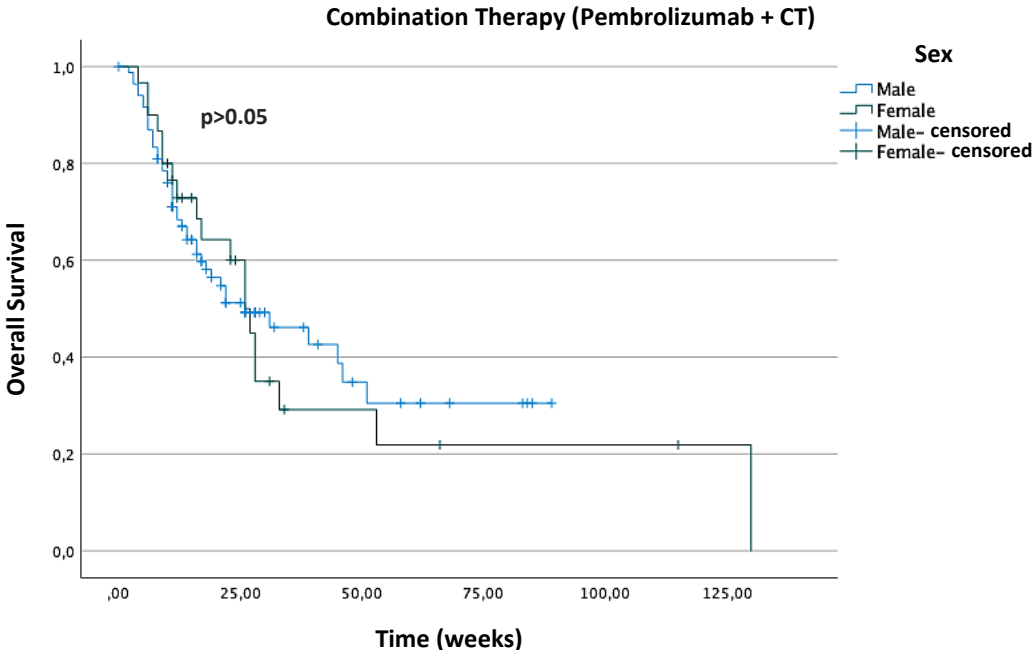

Figure 7 *- Kaplan–Meier analysis of overall survival in patients treated with Pembrolizumab + CT in combination therapy (Cohort #2) stratified by sex.*

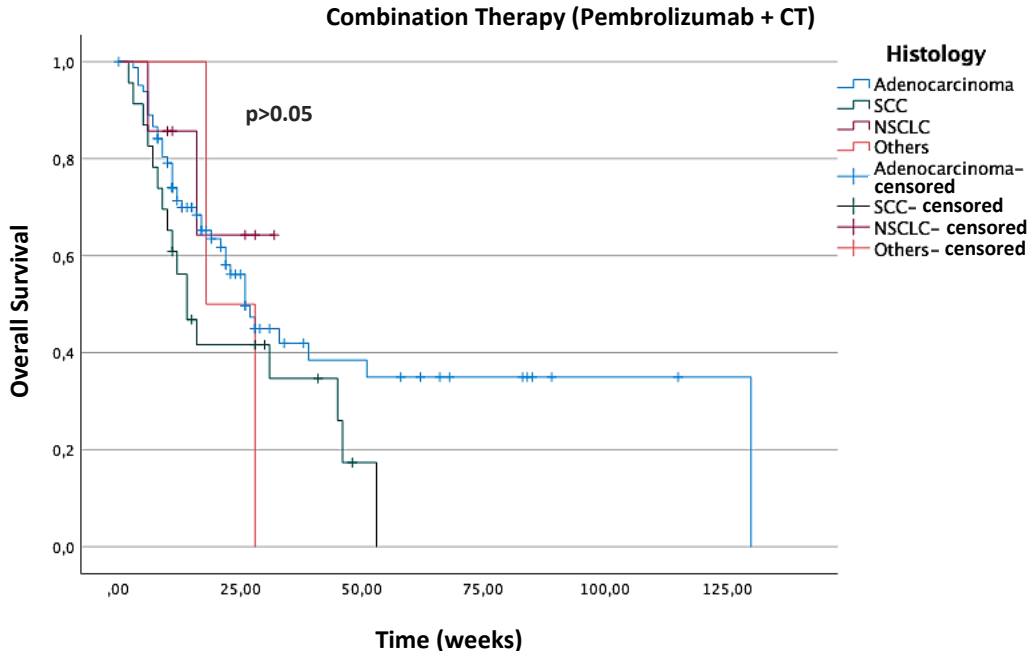

Figure 8 *- Kaplan–Meier analysis of overall survival in patients treated with Pembrolizumab + CT in combination therapy (Cohort #2) stratified by histology.*

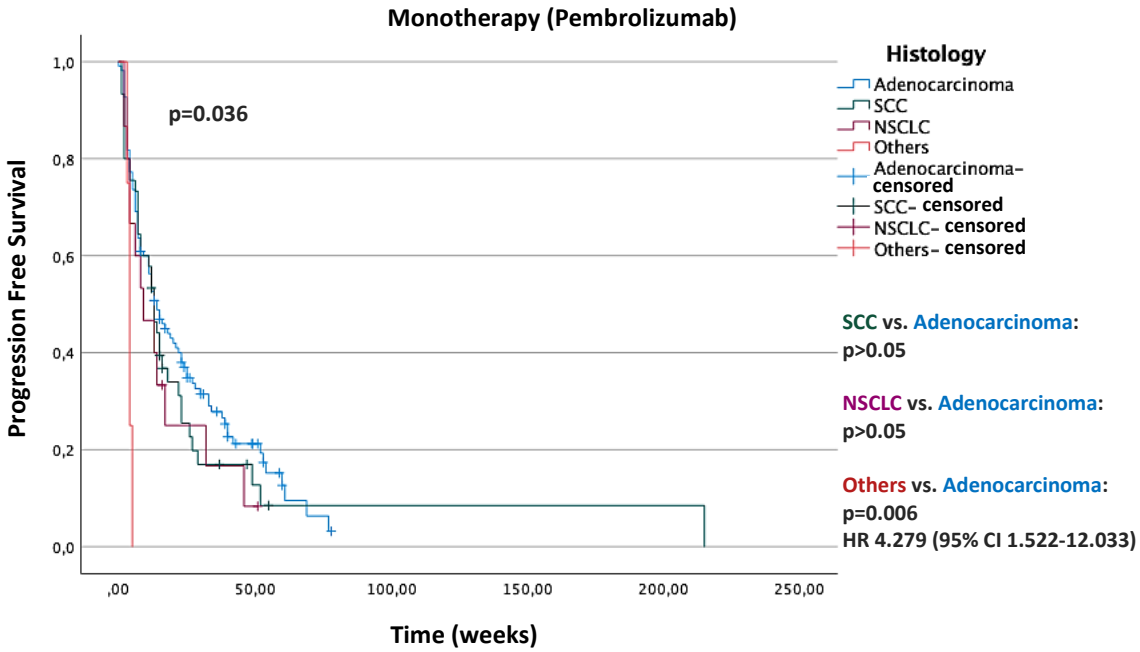

Figure 9 *- Kaplan–Meier analysis of progression-free survival in patients treated with Pembrolizumab in monotherapy (Cohort #1) stratified by histology.*

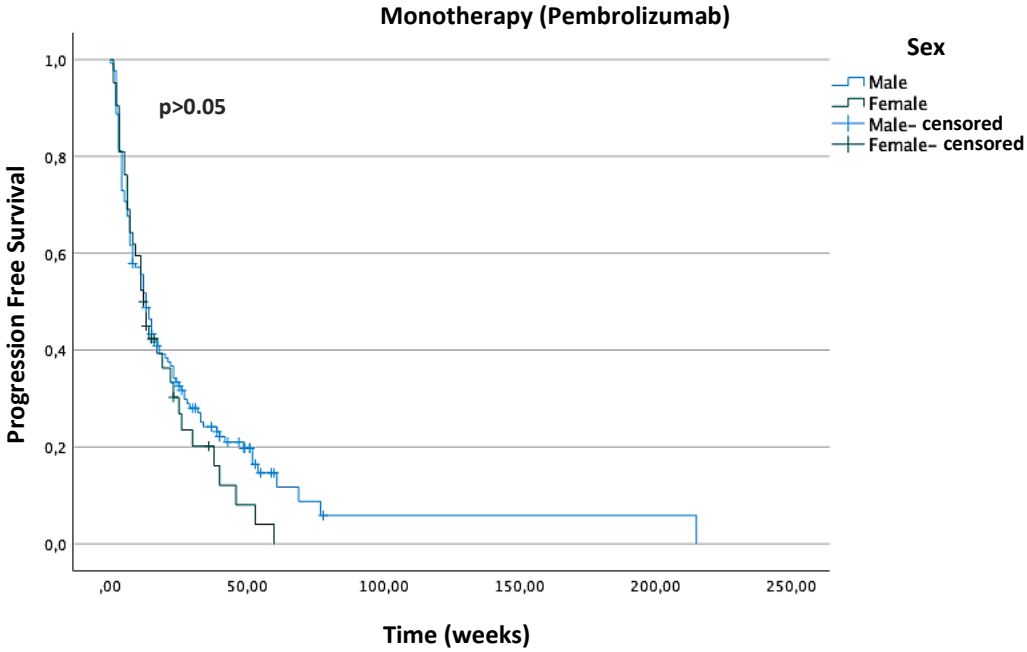

Figure 10 *- Kaplan–Meier analysis of progression-free survival in patients treated with Pembrolizumab in monotherapy (Cohort #1) stratified by sex.*

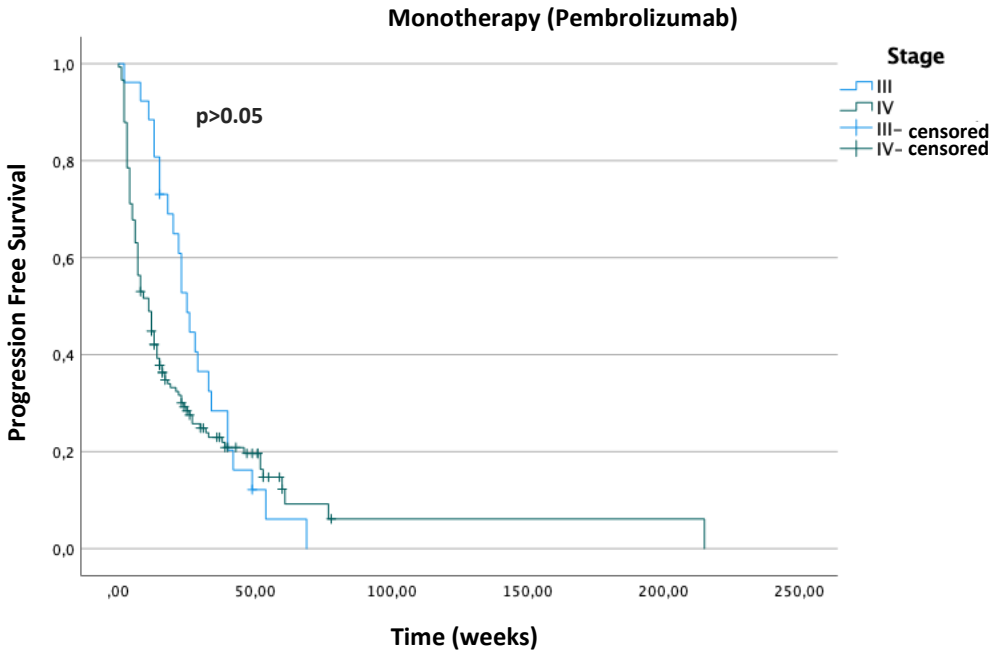

Figure 11 *- Kaplan–Meier analysis of progression-free survival in patients treated with Pembrolizumab in monotherapy (Cohort #1) stratified by stage.*

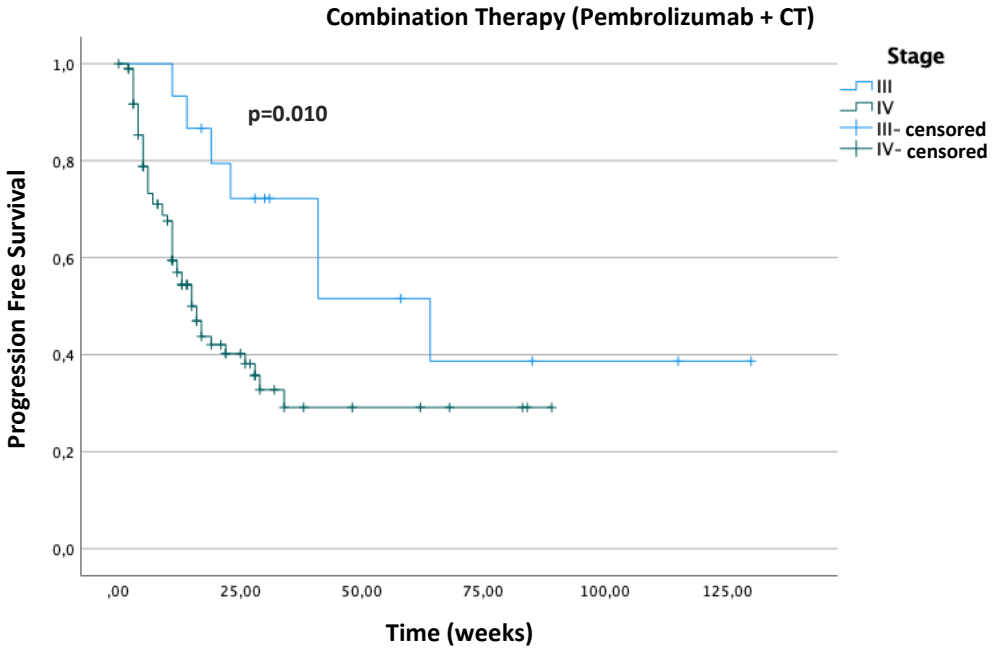

Figure 12 *- Kaplan–Meier analysis of progression-free survival in patients treated with Pembrolizumab + CT in combination therapy (Cohort #2) stratified by stage.*

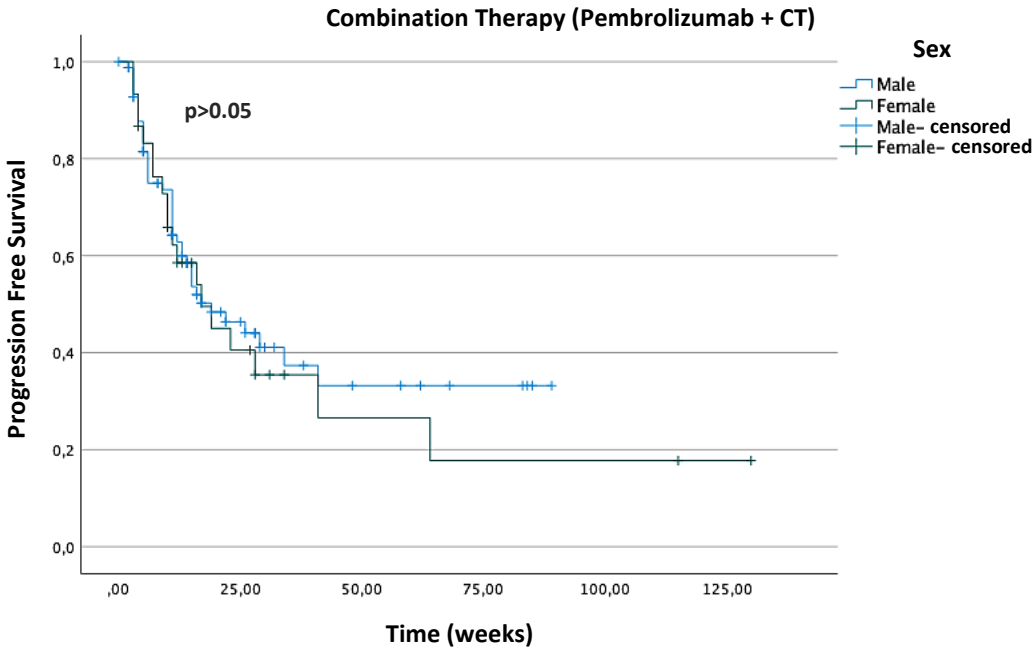

Figure 13 *- Kaplan–Meier analysis of progression-free survival in patients treated with Pembrolizumab + CT in combination therapy (Cohort #2) stratified by sex.*

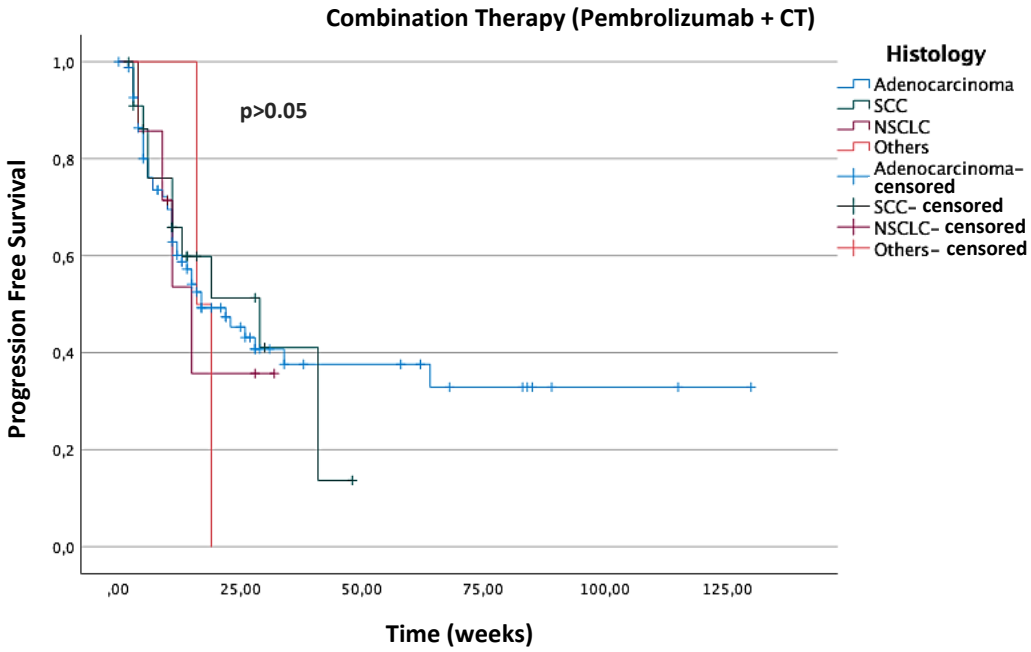

Figure 14 *- Kaplan–Meier analysis of progression-free survival in patients treated with Pembrolizumab + CT in combination therapy (Cohort #2) stratified by histology.*

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