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Clinical predictors of benefit with Pembrolizumab or Pembrolizumab plus chemotherapy in advanced, previously untreated, non-small cell lung cancer: a multicentric, retrospective study.

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Abstract

Background Pembrolizumab and Pembrolizumab-chemotherapy are two first line options for advanced, non-oncogene addicted NSCLC. Currently, PD-L1 is the only biomarker guiding physicians' choice, but it is often unsatisfying.

Methods This retrospective, multicentric study aims to assess the potential benefit from first line pembrolizumab +/- chemotherapy in pre-specified clinical (age, gender, PS ECOG, smoking history, histology, concomitant treatments, LDH-NLR stratified in three categories) radiological (number/type of metastatic sites, tumor burden), molecular (*KRAS*) subgroups of advanced NSCLC. Primary endpoint is OS. Prognostic factors were evaluated in a multivariable Cox regression model stratified per center. Interaction between treatment*features was assessed in a Cox regression model. OS and PFS were expressed through Kaplan-Meier curves, compared through log-rank test.

Results A total of 443 patients were included, 436 suitable for survival analysis (216 and 220 treated with pembrolizumab and combination, respectively).

Older age (p=0.03), PS ECOG≥2 (p<0.001), *KRAS*-mutant (p=0.02), LDH-NLR poor (p=0.03), tumor burden>102 mm (p=0.02), treatment with corticosteroids (p=0.02) and proton pump inhibitors (p=0.01) were independent, negative prognostic factors in the overall population.

OS was significantly improved by pembrolizumab in male (p=0.01), <68 years old (p=0.007), PS ECOG 0-1 (p=0.04), adenocarcinoma histology (p=0.01), *KRAS* wild type (p=0.03), with an interaction treatment*feature confirmed for age (p=0.04), PS ECOG (p<0.001), histology (p=0.007 for squamous and p=0.01 for other non-adenocarcinoma histology).

Conclusions Patients younger than 68, with PS ECOG 0-1 and adenocarcinoma histology might benefit from first line pembrolizumab, avoiding the exposure to chemotherapy. NLR-LDH stratification provides a new prognostic score, irrespectively of the addition of chemotherapy to pembrolizumab.

1.0 Introduction

1.1 Management of advanced, previously untreated, non-oncogene addicted non-small cell lung cancer

Lung cancer is the first cancer-related cause of mortality in men, and the third most frequent cause of cancer death in women, worldwide(1). The majority of lung cancers (80-90%) are non-small cell lung cancer (NSCLC) and the most common histology is the adenocarcinoma(1). Six molecular alterations have at least one matched targeted treatment that can be used as frontline strategy. However, tumors not harboring *EGFR* exon 19 deletions or exon 21 (L858R) mutations, *ALK/ROS1/RET* translocations, *BRAF*

V600E/*MET* exon14 skipping represent the majority of NSCLC(2). Treatment algorithm for them, and for tumors harboring *KRAS G12C* mutations, depends on patients' Performance Status ECOG (PS ECOG) and on the Programmed-Death Ligand-1 (PD-L1) expression levels(1).

With PD-L1≥50% and PS ECOG 0-2, patients may access to single agent immunotherapy, with either pembrolizumab, atezolizumab or cemiplimab. With PD-L1<50% and PS ECOG 0-1, patients may receive a combination of an immunotherapeutic agent (pembrolizumab or cemiplimab) with platinum based-chemotherapy. Other options in this setting include combinations of two immune-checkpoint inhibitors (ipilimumab plus nivolumab or durvalumab plus tremelimumab) with or without platinum-based chemotherapy.

The first immune-checkpoint inhibitor to be approved by the Food and Drug Administration (FDA)(3) and the European Medicines Agency (EMA)(4) in previously untreated, advanced/metastatic NSCLC was pembrolizumab, an anti-PD1 that achieved longer survival, compared to platinum-based chemotherapy, in tumors without *EGFR* mutations nor *ALK* translocations and PD-L1≥50%. Pembrolizumab was given at the dose of 200mg every two weeks for up to 35 cycles, or progressive disease (PD)/unacceptable toxicity. Median PFS (the primary endpoint) to first line pembrolizumab was 10.3 [95% confidence interval (95%CI) 6.7 – not reached (NR)] *vs* 6.0 (95% CI 4.2 – 62) months, in the chemotherapy arm[hazard ratio for disease progression or death (HR) 0.50 (95%CI 0.37 – 0.68), $p<0.001$](5). The five-years follow up update confirmed a statistically significant benefit in overall survival (OS), despite 66% of cross-over rate to pembrolizumab in the chemotherapy group: median OS was 26.3 (95%CI 18.3 – 40.4) with first line pembrolizumab *vs* 13.4 (95%CI 9.4 – 18.3) months with platinum-based chemotherapy (HR 0.62, 95%CI 0.48 – 0.81)(6).

The biological rationale for adding platinum-based chemotherapy to immunotherapy lies in the ability of several chemotherapy agents to induce the expression of PD-L1 on tumor cells; to modulate the tumor microenvironment, by reducing regulatory T cells (Tregs) and reprogramming of tumor-associated macrophages (TAMs); to promote immunogenic cell death, with subsequent presentation of neoantigens to immune cells(7).

Two phase III, randomized, double-blinded, controlled trials have been conducted in non-squamous (without *EGFR* sensitizing mutations/*ALK* translocations)(8) and squamous(9) metastatic, previously untreated NSCLC. In both studies, the addition of platinum-based chemotherapy to first line pembrolizumab improved survival outcomes compared to chemotherapy, independently of PD-L1 expression level.

In non-squamous histology, pembrolizumab 200mg every three weeks was associated to cisplatin/carboplatin plus pemetrexed for four cycles, and subsequently administered in maintenance with pemetrexed until disease progression, unacceptable toxicity or up to 35 cycles(8). In squamous histology, pembrolizumab 200mg every three weeks (or placebo) was administered with carboplatin plus

paclitaxel/[nab]-paclitaxel for four cycles and then continued as maintenance until progression, unacceptable toxicity or up to 35 cycles(9).

In both trials, the dual primary endpoints were OS and PFS. In non-squamous histology, median PFS was 8.8 (95%CI 7.6 – 9.2) months with pembrolizumab-chemotherapy *vs* 4.9 (95%CI 4.7 – 5.5) with placebo-chemotherapy [HR 0.52 (95%CI 0.43 – 0.54), $p<0.001$]. Median OS was NR in the pembrolizumab-combination arm and 11.3 (95%CI 8.7 – 15.1) months in the placebo-combination arm [HR 0.49 (95%CI 0.38 – 0.64), $p<0.001$](8). The five-year follow up confirmed the survival benefit of pembrolizumab-chemotherapy combination, over chemotherapy alone, with a five-year OS and PFS rate of 19.4% *vs* 11.3% and 7.5% *vs* 0.6%, respectively. Median OS was 22.0 (95%CI 19.5 – 24.5) months for pembrolizumab-chemotherapy and 10.6 (95%CI 8.7 – 13.6) months for chemotherapy alone. Effective crossover rate was 57%(10).

In squamous histology, median PFS was 6.4 (95% CI 6.2 – 8.3) months in pembrolizumab-combination *vs* 4.8 (95%CI 4.3 – 5.7) months in placebo-combination arm [HR 0.56 (95%CI 0.45 – 0.70), $p<0.001$]. Median OS was 15.9 (95%CI 13.2 – NR) months with pembrolizumab *vs* 11.3 (95%CI 9.5 – 14.8) months with placebo [HR 0.64 (95%CI 0.49 – 0.85), $p<0.001$](9). The five-year OS rate (18.4% vs 9.7%) confirmed the survival benefit of pembrolizumab-chemotherapy over chemotherapy alone, with a crossover rate of 51%(11).

Based on these results, international guidelines recommend platinum-based chemotherapy plus pembrolizumab in metastatic, previously untreated, non-oncogene addicted NSCLC, independently from PD-L1 expression, in both squamous and non-squamous histology(1,12). In the PD-L1≥50% subgroup, with a PS ECOG 0-1 and no contraindication to immunotherapy, clinicians should offer pembrolizumab monotherapy; in alternative, they may offer pembrolizumab-chemotherapy, according to the American Society of Clinical Oncology (ASCO) guidelines(12).

Currently, no validated biomarker, beyond PD-L1, exists to distinguish and choose between these two treatment strategies, particularly in the PD-L1≥50% population. The phase III, academic, prospective, controlled, randomized PERSEE trial (NCT04547504) aims to compare pembrolizumab-chemotherapy and pembrolizumab alone in patients with previously untreated, advanced NSLC, expressing PD-L1≥50% and without *EGFR* mutations/*ALK* translocations. This trial is currently ongoing(13).

1.2 *Single agent immunotherapy in challenging populations*

1.2.1 Clinical features

While single-agent immunotherapy has proven to be safe and effective, compared to chemotherapy, for advanced, previously untreated, non-oncogene addicted NSCLC, it must be noted that randomized controlled trials rarely mirror real world populations and might be unsatisfactory when it comes to assess the efficacy and safety of a therapeutic agent in "challenging" populations, often excluded or underrepresented in clinical trials(14).

Some evidence suggests that the interplay between hormones, genes, microbiome and environment might cause gender-related differences in immune responses and immunotherapy efficacy(15,16). Specifically, male patients might achieve better responses to immunotherapeutic agents, as it has been demonstrated across different solid tumors. A meta-analysis conducted on raw exome/transcriptome data, for a total of 1008 patients treated with immune-checkpoint inhibitors, revealed a significant association between male gender and higher rate of response to treatment [odds ratio (OR) 1.22, 1.03 – 1.43, p=0.019]. In this cohort, 76 patients (7.5%) received immunotherapy for the treatment of NSCLC(17).

Conforti *et al* conducted two systematic reviews and meta-analyses to assess sex-dimorphism in response to immunotherapy NSCLC. The first one included six randomized controlled trial of immune-checkpoint inhibitors administered alone or in combination with chemotherapy. Its aim was to test the interaction between the immunotherapeutic agent, with or without chemotherapy, and gender. The results indicated that, while men might benefit from anti-PD-(L)1 alone, compared to chemotherapy, female patients might achieve higher benefit from combination therapy. Indeed, the pooled OS-HR was 0.78 (95%CI 0.60 – 1.00) and 0.97 (95% CI 0.79 -1.19) in men and women, respectively, with anti-PD-(L)1 single agent *vs* chemotherapy; 0.76 (95%CI 0.64 – 0.91) and 0.44 (95% CI 0.25 – 0.76) with combined therapy *vs* chemotherapy. Overall, the difference between the two treatment strategies, according to gender, was statistically significant (p=0.002)(16).

The second systematic review and meta-analysis focused on patients with previously untreated NSCLC and high expression of PD-L1(≥50%)(15). The primary endpoint was the efficacy of the anti-PD-(L)1 *vs* chemotherapy in men and women. The study included four randomized controlled trials. While men seemed to have benefit from single agent immunotherapy (pooled OS-HR= 0.59, 95%CI 0.50 – 0.69), the same result is not observed in female patients (pooled OS-HR= 0.84, 95%CI 0.64 – 1.10). Overall, the benefit with anti-PD-(L)1 monotherapy was statistically significant higher in men than in women (p=0.04)(15).

A meta-analysis of 25 randomized controlled trials of anti-PD-(L)1 with or without anti-CTLA4 and/or chemotherapy, and platinum-based chemotherapy as control arm, in previously untreated, advanced NSCLC explored the differential efficacy of immunotherapy alone and immunotherapy plus chemotherapy in clinical/molecular subgroups. Interestingly, immunotherapy-chemotherapy improved PFS and OS in female patients, compared to immunotherapy alone: HR for PFS was 1.65 (95%CI 1.25 – 2.18), p<0.001; HR for OS was 1.31 (95%CI 1.01 – 1.71), p=0.04(18).

To note, female patients represented the 40% and 37% of patients included in the experimental (pembrolizumab) and standard (chemotherapy) arm of Keynote 024, respectively(5).

Clinical trials for immunotherapy in NSCLC often have limited representation of patients with no history of smoking(5,10,11). This is because lung cancer in individuals who have never smoked or have minimal smoking history is typically associated with oncogene addiction, such as *EGFR* sensitizing mutations or *ALK/ROS1* translocations, and therefore requires distinct treatment approaches(2). Patients with no smoking history tend to develop tumors with lower tumor mutational burden (TMB)(19) and accumulating evidence demonstrates that the tumor microenvironment in patients with no history of smoking differs from that of current or former smokers. Indeed, tumor microenvironment in patients with no smoking history appears to be characterized by an immunosuppressive status, with reduced and impaired levels of cytotoxic cells and pro-tumoral effects exerted by TAMs with M2 phenotype(20). An unsupervised hierarchical clustering analysis of 75 genes linked to cytotoxic T lymphocytes activation, conducted on resected NSCLC samples, showed a statistically significant difference in the activation level of cytotoxic infiltrating lymphocytes between smokers and never/ever smokers(21). Taken together, this evidence suggests that single agent immunotherapy might be unsatisfactory in patients with NSCLC and no smoking history.

Data on safety and efficacy of immunotherapy in patients with PS ECOG≥2 derives mostly from real world studies, since eligibility criteria for randomized controlled trials are usually restricted to PS ECOG 0-1(5,9–11). A multicenter, retrospective, observational Italian study collected data from patients with PS ECOG of 2 and PD-L1≥50% receiving first line pembrolizumab. The primary endpoint of the study was the six-months progression-free survival rate. This study showed that the only independent factor impacting survival to first line pembrolizumab in patients with advanced NSCLC and PS ECOG of 2 is the cause of the frailty: patients considered as “PS ECOG 2” because of comorbidities achieved higher benefit from immunotherapy compared to patients who were considered frail because of cancer burden(22). Recently, the IPSOS trial compared first line atezolizumab (anti-PD-L1) to single agent chemotherapy in patients unfit for platinum-based chemotherapy and, thus, not represented in immunotherapy pivotal trials. The study included 246 (81%) and 122 (88%) patients with PS ECOG≥2 (81%) in the atezolizumab and in the chemotherapy arms, respectively. The remaining patients were considered ineligible for platinum-based chemotherapy because of age (≥70 years old) and comorbidities. The primary endpoint was OS in the intention-to-treat population. Median OS to first line atezolizumab was 10.3 (95%CI 9.4 – 11.9) months vs 9.2 (95%CI 5.9 – 11.2) to single agent chemotherapy [HR 0.78 (95%CI 0.63 – 0.97), p=0.028](23).

Brain and liver metastases are considered immune-privileged sites and, thus, challenging to be controlled by immunotherapy. As regards central nervous system, the recent discovery of lymphatic vessels lining the dural sinuses challenges the foundation for a fresh perspective on the brain as an immune-privileged anatomical region(24). Moreover, damages caused by radiotherapy for local control, and by the

development of brain metastases themselves, might cause a disruption of the blood brain barrier, thus facilitating the arrival of compounds(25).

Brain metastases are present, at diagnosis, in about 10-20% of NSCLC, according to real-world data (26), and result in poor prognosis(27). Typically, randomized controlled trials limit eligibility to patients who have asymptomatic and/or well-controlled brain metastases following local treatment. Thus, in Keynote 024, 189 and 407, patients with brain metastases represented a small percentage of the intention-to-treat population: 28 (9%), 108 (17,5%), 43 (8%) patients in each study, respectively(5,10,11).

The expanded access program (EAP) of nivolumab in Italy included patients with metastases if asymptomatic, neurologically stable, off-corticosteroids or in stable/decreasing dose ≤10mg/die of prednisone. Nivolumab was administered at the dose of 3mg/kg, every two weeks, in subsequent lines. Out of 1588 patients, 409 (26%) had brain metastases before the start of nivolumab. Median PFS was 3.0 (95%CI 2.7 – 3.3) months and median OS from the start of nivolumab was 8.6 (95%CI 6.4 – 10.8) months(27). In a phase II trial of patients with melanoma or NSCLC and untreated or progressive brain metastases, intracranial response was achieved by four out of 18 (22%) and six out of 18 (33%) patients with melanoma and NSCLC, respectively(28).

Data regarding the association of ipilimumab (anti-CTLA-4) and nivolumab and its intracranial efficacy will not be discussed here since it is a double immunotherapy combination strategy.

The presence of liver metastases is an independent predictor of worse survival outcomes with immunotherapy in solid tumors(29,30), and both preclinical studies and clinical experiences show that liver-related immune-tolerance diminish sensitivity to immune-checkpoint inhibitors. In mice models, liver metastases seem to induce CD8+ T cell depletion: indeed, in the liver, Fas+ CD8+ T cells bind Fas ligand expressed on immunosuppressive monocyte-induced macrophages, leading to apoptosis of CD8+ T cells and, ultimately, to an immune desert microenvironment(30).

1.2.2. Concomitant treatments

Patients receiving corticosteroids at a dose of ≥10mg of prednisone equivalent were specifically excluded from pivotal trials of first line immunotherapy(5,31,32) or immunotherapy-chemotherapy(8,9,33) for advanced, non-oncogene addicted NSCLC. Real world data are consistent in suggesting that the use of corticosteroids before treatment start impairs response and survival outcomes to immune-checkpoint inhibitors(34,35). However, the cause leading to the need of steroid treatment (cancer-related symptoms or different conditions) might be determinant: Ricciuti *et al* demonstrated that, when administered for cancer-unrelated reasons, corticosteroids do not impair PFS and OS to immune-checkpoint inhibitors. Specifically, when comparing patients receiving ≥10mg of prednisone equivalent for cancer burden, patients receiving ≥10mg of prednisone equivalent for cancer-unrelated conditions and patients receiving none or <10mg of

prednisone equivalent, median PFS was 1.4 *vs* 4.6 *vs* 3.4 months, respectively ($p < 0.001$ across the three groups); median OS was 2.2 *vs* 10.7 *vs* 11.2 months, respectively ($p< 0.001$ across the three groups) (36).

Proton pump inhibitors and antibiotics might alter the composition of gut microbiota and thus negatively influence immune-sensitivity(37–39). A pooled analysis of data from the phase II POPLAR (atezolizumab) and the phase III OAK (atezolizumab *vs* docetaxel) trial showed that, among patients undergoing immunotherapy, those who were also prescribed antibiotics or proton pump inhibitors experienced a significantly shorter OS: 8.5 versus 14.1 [HR 1.32 (95% CI 1.06–1.63), $p= 0.01$] months for antibiotics and 9.6 versus 14 [HR 1.45 (95% CI 1.20–1.75), $p= 0.0001$] months for proton pump inhibitors, respectively. On the contrary, no associations between these concomitant medications and OS emerged in the chemotherapy cohort(37). Consistently, real world studies showed that the use of antibiotics reduces OS in patients with NSCLC treated with immune-checkpoint inhibitors [HR 2.5 (95%CI 1.6–3.7), $p<0.01$](38).

To note, a multicentric, retrospective study demonstrated that concomitant antibiotics do not affect the outcomes to chemo-immunotherapy, in contrast to what had been reported for single agent immunotherapy(40). Thus, this research will not investigate the differential efficacy of immunotherapy alone or in combination with chemotherapy in patients with advanced, non-oncogene addicted NSCLC exposed to antibiotics.

1.2.3 Tumor burden

Accumulating preclinical and clinical evidence suggest that a large tumor burden might impair immune response through two main mechanisms linked to cancer cells metabolism:

1) Through the overexpression of GLUT transporters, cancer cells compete with T cells for glucose uptake.
2) Cancer cell metabolism, based on aerobic glycolysis, produces lactate, which, when released in tumor microenvironment, impairs the activity infiltrating CD8+ cells, but it is efficiently used by Tregs.

Moreover, chronic inflammation and/or cancer are linked to an immune-senescence profile, with larger tumors corresponding to a higher grade of senescent phenotype(41).

Tumor burden can be assessed through imaging-based and non-imaging-based methods: Response Evaluation Criteria Ins Solid Tumors (RECIST) 1.1 measurement of CT scans images(42), 2- deoxy-2-[18F]- fluoro-d- glucose (FDG)- PET, quantification of circulating tumoral DNA (ctDNA) and/or circulating tumor cells (CTCs), blood LDH.

The role of ctDNA and CTCs in reflecting tumor burden extension will not be further discussed in this work since this research focus on radiological and biological markers.

In clinical trials, tumor burden is measured using RECIST 1.1. RECIST criteria guarantee a standardization of measurements, but they come with some limitations: some lesions are not measurable, either because their dimensions are under the threshold considered for significance by RECIST 1.1, either for their peculiar characteristic (i.e., pleural/pericardial effusion, lymphangitis, osteolytic bone metastases). Thus, RECIST criteria might underestimate the tumor burden and the impact it can have on anti-cancer immunity(41). On the contrary, FDG-PET enables the assessment of a wider range of lesions and, most importantly, permits to correlate the morphology with the metabolic activity, by the calculation of metabolic total volume (MTV)(41). At present, the CT scan is the most commonly used radiological method for the staging and reevaluation of metastatic lung cancer, and it is recommended by international guidelines(1), thus we assessed tumor burden using RECIST 1.1 criteria applied to radiological images obtained with CT scan.

Data from randomized controlled trials and retrospective studies, in melanoma or NSCLC patients, are overall concordant in showing that a larger tumor burden, defined through different cut-offs, is associated with worse survival outcomes. A post-hoc analysis of Keynote-001, a study of pembrolizumab in patients with advanced melanoma, found as median baseline tumor size 102mm, which was used as a threshold to define "large" and "small" tumor burden. In the multivariable analysis, "small" tumor burden was associated with longer OS (HR 0.61, $p < 0.001$)(43). Similarly, a pooled analysis of patients with advanced NSCLC treated with atezolizumab in four clinical trials showed that patients with a sum of the longest diameters under the median (64mm) had significantly increased overall survival compared to patients with larger baseline tumor size [16.0 *vs* 10.0 months, HR 1.64 (95%CI 1.41 – 1.91), $p<0.001$](44). Retrospective studies conducted in patients with advanced NSCLC treated with anti-PD-(L)1 are generally consistent with the previously reported result(45,46). However, in one retrospective study, baseline tumor size (median value 64mm) was not associated with PFS, nor with OS(47).

LDH is an enzyme whose elevated serum levels depends on intratumor hypoxia and production of lactic acid(48). Thus, its levels tend to increase when the tumor size is bigger(43,48). Several studies identify serum LDH levels over the upper limit of normal as a negative prognostic factor and a predictor of reduced efficacy of immune-checkpoint inhibitors in solid tumors. In a study of patients with advanced/metastatic tumors treated with anti-PD-(L)1 in phase I-III trials, the presence of liver metastases [HR, 1.69 (95%CI 1.03 - 2.79), $p= 0.04$) and high level of serum LDH [HR, 1.002 (95% CI 1.001 - 1.002), $p<0.001$] were independent prognostic factors (in a sub-cohort constituted by non-melanoma patients)(29). A meta-analysis of six studies, for a total of more than 1000 patients with advanced NSCLC, showed that pre-treatment LDH serum levels influence survival outcomes to immune-checkpoint inhibitors: elevated LDH was associated with poor PFS [HR 1.62 (95% CI 1.26-2.08), $p<0.001$] and OS [HR 2.38 (95% CI 1.37-4.12), $p=0.002$](49).

Neutrophil-to-lymphocyte ratio is associated with poor outcomes with immunotherapy(50–52) and, when ≥3, it is independently associated with primary resistance to immune-checkpoint inhibitors, as defined per Society for Immunotherapy of Cancer (SITC) criteria(30). NLR might be influenced by corticosteroids

administration: in a study of 147 patients treated with immunotherapy and for whom baseline blood samples were available, NRL was higher in patients taking concomitant corticosteroids rather than other patients (6.9 *vs* 3.4, $p<0.001$), and this modulation of peripheral blood cells seemed to be maintained at four (6.9 *vs* 3.4, $p<0.001$) and six (4.0 *vs* 2.2, $p<0.001$) weeks(35).

1.2.4 Molecular features

KRAS mutations are the most common molecular alterations in NSCLC, being present in around 25% of adenocarcinomas(53,54). The presence of a *KRAS* mutation and a concomitant alteration in the oncosuppressors *Serine/threonine kinase 11 (STK11)/Kelch-like ECH-associated protein 1 (KEAP1)* identify unique subgroups of patients unlikely to respond to PD-1 axis blockade(55,56). In a cohort of 174 patients with *KRAS*-mutant NSCLC treated with anti-PD-(L)1 +/- anti-CTLA4, overall response rate (ORR) was significantly different in the presence of a comutation in *STK11/KEAP1* (7.4%), *tumor protein 53 (TP53)* gene (35.7%), or no comutation identified (28.6%, $p<0.001$ Fisher exact test)(55). PFS differed among the three subgroups ($p=0.0018$), being significantly shorter in the presence of *STK11/KEAP1* comutations [HR 1.77 (95%CI 1.16–2.69), $p= 0.0072$ compared to *TP53*-comutant; HR 1.98 (95% CI 1.33–2.94), $p<0.001$ compared to *KRAS*-mutant], whereas no difference was seen between *TP53*-comutant and *KRAS*-mutant subgroups. Similarly, OS outcomes differed between the subpopulations, with *STK11/KEAP1*-comutants achieving shorter survival: median OS was 6.4 months *vs* 16.0 and 16.1 months in *TP53*-comutant and *KRAS*-mutant (p=0.0045)(55).

To note, the presence of *STK11/KEAP1* mutations impaired response and survival outcomes to anti-PD-(L)1, irrespective of the presence of a *KRAS* concurrent mutation, in non-squamous carcinoma with PD-L1≥1 [ORR 0% vs. 34.5%, $p= 0.026$; PFS HR 4.76 (95% CI 2.0–11.1), $p= 0.00012$; OS HR 14.3 (95% CI 3.4–50.0) $p<0.0001$](55).

A recent study suggests that the presence of *STK11/KEAP1* mutations leads to a different immune profile, in terms of gene expression and cells infiltration, in *KRAS*-mutant, but not in *KRAS*-wild type tumors(57).

Indeed, in patients with *KRAS*-wild type tumors, PFS and OS were similar between *STK11*-mutant and wild type: PFS was 2.5 (95%CI 2.1 – 3.6) and 2.8 (95%CI 2.5 – 3.2) months [HR 0.92 (95%CI 0.75 – 1.14), $p=0.45$] and OS 13.0 (95%CI 7.7 – 16.2) and 12.4 (95%CI 11.0 – 14.1) months [HR 1.1 (95%CI 0.88 – 1.38), p=0.45] in *STK11*-mutant and wild type, respectively. On the contrary, in *KRAS*-mutant tumors, the presence of a concurrent *STK11* mutations led to significantly worse survival outcomes: PFS was 2.0 (95%CI 1.7 – 2.3) and 4.8 (95%CI 3.7 – 6.2) months, in *STK11*-mutant and non-mutant, respectively [HR 2.04 (95%CI 1.66 – 2.51), $p<0.0001$]; OS was 6.2 (95%CI 4.4 – 9.2) and 17.3 (95%CI 15.1 vs 22.8) months, in STK11-mutant and non-mutant, respectively [HR 2.09 (95%CI 1.68 – 2.61), $p<0.0001$](57). Similar results are reported for *KEAP1* mutations, with worse PFS and OS in *KRAS*-mutant cases and a significant association with shorter PFS ($HR = 2.15, p < 0.0001$) and OS ($HR = 2.44, p < 0.0001$) in *KRAS*-

mutant subgroup in multivariable analysis(57). Through a hierarchical gene ontology analysis, signaling pathway related to MHC class II protein complex, T-cell activation, immune response-activating signaling, leukocyte migration, leukocyte degranulation, and myeloid leukocyte activation resulted down-regulated in tumors which harbored both *KRAS* and *STK11* mutations, compared to tumors harboring only the *KRAS* mutation. Through the same analysis, signaling pathways implied in external side of plasma membrane, regulation of T-cell activation, T-cell receptor signaling, defense response to virus, regulation of leukocyte cell-to-cell adhesion, and lymphocyte migration resulted upregulated in *KRAS*-mutant tumors, compared to *KRAS*/*KEAP1*-mutant tumors(57). Similarly, cells infiltration in tumor microenvironment differed according to molecular status, with an over-representation of M1 macrophages ($p<0.01$), M2 macrophages ($p< 0.01$), granulocyte-monocyte progenitors ($p=0.02$), CD4+ effector memory cells ($p=0.01$), and B cells (58)same differential infiltration was not seen in tumors wild type for both *KRAS* and *STK11* when compared to *KRAS*-wild type/*STK11*-mutant tumors. Moreover, *KRAS*-mutant/*STK11*-mutant tumors were more infiltrated with neutrophils ($p < 0.01$), compared to only *KRAS*-mutant tumors(57). CD8+ T cells ($p< 0.001$), CD8+ central memory T cells ($p<0.01$), CD8+ naive T cells ($p=0.02$), and B cells ($p=0.01$) were significantly over-represented in tumors harboring only a mutation in *KRAS*, compared to tumors harboring a concomitant mutation in *KEAP1*. This difference was not seen between *KRAS*-wild type tumors with or without *KEAP1* mutations. MSC were significantly enriched in tumor harboring both *KRAS* and *KEAP1* mutations, compared to tumors harboring only KRAS mutation ($p = 0.02$)(57).

To note, the presence of co-occurring mutation in *STK11, KEAP1, SMARCA4* appears to impair response and survival outcomes even when chemotherapy is added to immunotherapy. In a retrospective analysis of 1285 patients treated with combination therapy, mutations in *STK11*/*KEAP1*/*SMARCA4* genes were associated with significantly worse ORR, PFS, and OS (all $p < 0.05$). The same was observed for tumors harboring a *KRAS* mutation and concurring alterations in one of these genes (all $p<0.05$). In the *KRAS* wild-type subgroup, *KEAP1*/*SMARCA4* mutations were associated with worse PFS and OS (all $p < 0.05$), whereas *STK11* mutational status seemed not to impact neither PFS ($p = 0.16$) nor OS ($p = 0.38$)(58).

Overall, both *STK11* and *KEAP1* arise as independent factors of resistance to immunotherapy in *KRAS*-mutant tumors. However, since mutations in *STK11* and *KEAP1* appear to lead to poor outcomes, regardless of the administered treatment, they might be negative prognostic factors, rather than predictive ones(58,59).

The role of *KRAS* mutational status, irrespective of the presence of co-mutations, as a predictive factor for immunotherapy has not been validated, yet.

1.3 The (limited) role of PD-L1 as a predictive factor for immunotherapy

As aforementioned, PD-L1 expression level is the only predictive factor guiding (and limiting) pembrolizumab and pembrolizumab-chemotherapy prescription for first line treatment of advanced, non-oncogene addicted NSCLC(1).

PD-L1 assessment comes with some limitations, first from a technical point of view. PD-L1 expression is assessed on tumor cells (tumor proportional score – TPS) through immunohistochemistry (IHC) and expressed as a percentage. The assessment is reliable if at least 100 tumor cells are found in the sample(60). However, diagnosis of lung cancer, especially in the advanced stage, often relies on small biopsies, making the paucity of tissue an issue that it is difficult to overcome(60,61). As regards cytological samples, a real-world experience showed a numerically higher inadequacy rate (14.58%) of cytological samples compared to histological samples (6.75%), but the difference was not statistically significant (p=0.08)(62).

IHC for PD-L1, on histological samples, should be performed on formalin-fixed/paraffin-embedded (FFPE) freshly cut tissue sections at a thickness of 3–5 μM and mounted on positively charged slides, and a positive control should always be provided. Different steps in the pre-analytical process, such as fixation, fixation time and sample processing, might affect the technique exploitation and the final reading(60,62). Moreover, several antibodies are available for PD-L1 testing, and, despite some harmonization studies have been ruled out, no conclusion has been reached so far(60).

Assuming that PD-L1 is tested in baseline tissue samples, as recommended for the workup of advanced NSCLC(2), and, therefore, not considering the temporal variability, the site of biopsy might still be a critical issue. For instance, accumulating evidence show that tumor microenvironment of liver metastases differs in immune profile and cellular infiltration compared to the primary tumors(63).

When it comes to interpreting the results and ensuring reproducibility in scoring, it is important to acknowledge that, currently, this is entirely observer-dependent, although some attempts in IHC reading automatization through digital pathology have been ruled out(64). However, this does not represent the clinical practice right now.

Finally, it must be noted that, while PD-L1 is a discriminant for choosing frontline treatment strategy, nivolumab had been approved in subsequent lines irrespective of PD-L1 expression, based on improved OS compared to docetaxel(65). Similarly, atezolizumab was approved as subsequent treatment in previously treated NSCLC based on the results of the phase III OAK study, regardless of PD-L1 expression(66), and pembrolizumab was approved in the same setting with the cut off of ≥1%, based on the results of the phase III Keynote-010 study(67).

2.0 Patients and methods

This is a retrospective, multicentric study aiming to assess the potential benefit from pembrolizumab as single agent or in combination with platinum-based chemotherapy in pre-specified subgroups of previously untreated patients affected by advanced, NSCLC. Primary outcome is survival, and the primary endpoint is OS. PFS is a secondary endpoint.

Adult patients who received at least one dose of pembrolizumab alone or pembrolizumab plus platinum-based chemotherapy for advanced/metastatic non-oncogene addicted NSCLC were considered eligible for the study. The presence of *EGFR* common mutations (exon 19 deletions/L858R in exon 21), *ALK* translocations or *ROS1* translocations were key ineligibility criteria.

Clinical, radiological, molecular data were collected from patients treated with first line pembrolizumab or pembrolizumab plus chemotherapy in three European facilities between 2017 and 2023.

Molecular alterations were researched in tissue or blood samples, with different molecular biology techniques and panels according to the center, and the year of diagnosis.

Baseline NLR and LDH were categorized into good, intermediate, and poor groups based on NLR≥3 and LDH>upper limit of normal, with none, one, or both criteria met.

Pre-specified subgroups included:

- Age (categorized according to the median value).
- Gender.
- No smoking history.
- Histology (adenocarcinoma/non-adenocarcinoma).
- PS ECOG (0-1/≥2).
- Presence of brain metastases at baseline.
- Presence of liver metastases at baseline.
- Number of metastatic sites (according to the median value) at baseline.
- Tumor burden≥64 mm (defined per RECIST 1.1). This threshold derives from a pooled analysis of patients with advanced NSCLC treated with atezolizumab in four clinical trials: patients with a sum of the longest diameters under the median (64mm) had significantly increased overall survival compared to patients with larger baseline tumor size(43).
- Tumor burden>102 mm (defined per RECIST 1.1). This threshold derives from an analysis of Keynote-001, a study of pembrolizumab in patients with advanced melanoma: tumor burden under this cut off was independently associated with better OS in the multivariable analysis(43).
- *KRAS* molecular status.
- Concomitant treatment (e.g., ongoing at ICI start) with corticosteroids (equivalent to prednisone≥10mg).

- Concomitant treatment (e.g., ongoing at ICI start) with proton pump inhibitors.

As previously mentioned, the effectiveness of pembrolizumab or a combination of pembrolizumab with chemotherapy was studied in both *KRAS*-mutant and *KRAS*-wild type tumors. However, the study did not consider the presence of concomitant alterations in *STK11, KEAP1, TP53*, because a comprehensive molecular analysis, including these mutations, was only available at the time of diagnosis for approximately one third of the study population.

Data cut-off for the presented results was 31st August 2023.

At the time this study was conducted, pembrolizumab-chemotherapy was approved and reimbursed in Italy for patients with PD-L1 expression levels below 50%(68), whereas there were no restrictions on prescription of pembrolizumab-chemotherapy based on PD-L1 levels in France. Single-agent immunotherapy was employed only with PD-L1 expression ≥50% in both countries(4).

2.1 *Statistical analysis*

Patients with at least six months of follow-up were considered suitable for survival analysis. PFS has been calculated from the date of start of first line treatment to the date of progression of disease or death. OS has been calculated from the date of start of first line treatment to the date of death from any cause or the date of last follow up. PFS and OS curves have been estimated through the Kaplan-Meier method and compared through log-rank test. The interaction between the treatment and different clinical, radiological, molecular features has been tested through a Cox regression model.

Multivariable analysis for OS has been conducted through Cox regression model. Known prognostic factors (age, gender, PS ECOG at diagnosis, histology, presence of brain metastasis and liver metastasis at diagnosis, number of metastatic sites at diagnosis, tumor burden≥64 mm and >102mm, concomitant treatment with corticosteroids or proton pump inhibitors), NLR/LDH categories, *KRAS* molecular status, and the treatment were tested in univariable analysis. Factors which resulted associated with a $p<0.05$ in the univariable analysis, were included in the multivariable model. The model was stratified per treating center.

The association between binary categorical variables has been estimated through the Fisher exact test. The association between non-binary categorical variables has been estimated through the Chi-square test.

Statistical significance was defined as a two-sided p value < 0.05. Statistical analysis has been conducted in R software version 4.2.2.

3.0 Results

A total of 443 patients were included; 436 were suitable for survival analysis, 216 treated with pembrolizumab and 220 treated with combination therapy. The flowchart of the included patients is shown in Figure 1.

Median age was 68 (interquartile range – IQR 61-73) years. Most patients were male (66%, n=292), with previous or current smoking history (92%, n=403). Baseline characteristics are gathered in Table 1.

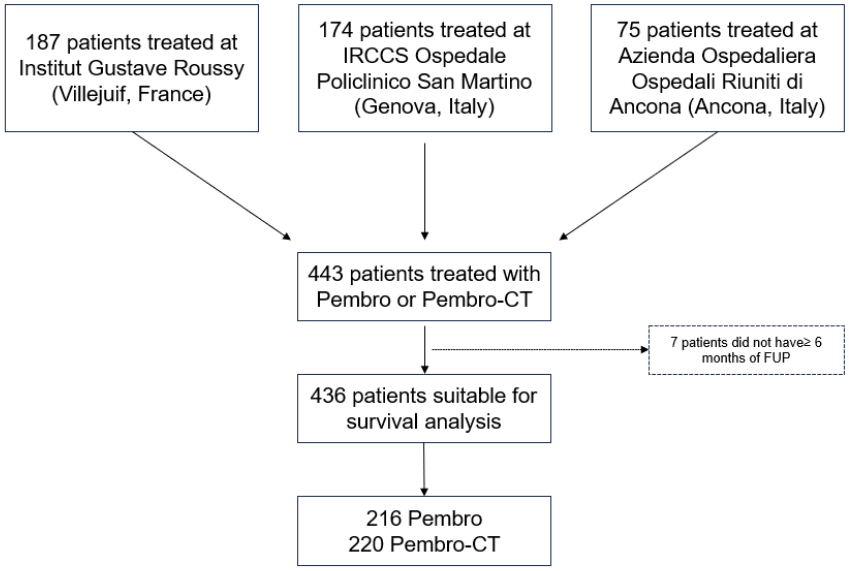


Figure 1 Flowchart of included patients

Older patients tended to be treated with pembrolizumab (p=0.03 when age was considered as a continuous variable and p=0.0006 when it was categorized according to the median value). Patients with tumors of non-adenocarcinoma histology tended to be treated with pembrolizumab alone (p=0.002). Notably, patients with squamous histology tended to be older (p=0.0000003) and 47% of patients with non-adenocarcinoma histology tumors tended to have a PS ECOG≥2 at diagnosis (p=0.004).

Patients with at least three metastatic sites tended to be treated with combination therapy (p=0.01), as well as patients with a stage IV disease (p=0.02).

Among patients treated with combination therapy, 29 (13%) had a PD-L1≥50%. Notably, in this subgroup, 22 (76%) patients had at least three metastatic sites, 15 (52%) had brain and 6 (21%) had liver metastases at diagnosis. As regards other baseline characteristics in this subgroup, median age was 63 years old (IQR 58-66), 18 (62%) patients were male; 27 (93%) had smoking history and 24 (83%) had intermediate or poor NLR/LDH; PS ECOG was <2 in 17 (59%) cases; seven (24%) patients were under corticosteroids >10mg

and seven (24%) patients were under proton pump inhibitors treatment at the time of diagnosis; 20 (69%) tumors had adenocarcinoma histology and *KRAS* was mutated in 13 (45%) cases.

	Immunotherapy N=219	Chemo-immunotherapy N=224	*p*
Age			
Median (IQR)	70 (63-75)	65 (59-71)	<0.001
Gender			
Male	141 (64%)	151 (67%)	
Female	78 (36%)	73 (33%)	0.5
Smoking history			
Current	65 (30%)	84 (38%)	
Former	140 (60%)	115 (51%)	
Never	14 (6.4%)	23 (10%)	
Unknown	0 (0%)	2 (0%)	0.026
PS ECOG			
0-1	169 (77%)	176 (79%)	
≥2	50 (23%)	48 (21%)	0.7
Histology			
Adenocarcinoma	146 (67%)	182 (81%)	
Squamous carcinoma	44 (20%)	23 (10%)	
Other	29 (13%)	19 (8.5%)	0.002
Stage at diagnosis			
IV	196 (89%)	216 (96%)	
IIIC	8 (3.7%)	2 (0.9%)	
IIIB	8 (3.7%)	2 (0.9%)	
IIIA	1 (0.5%)	0 (0%)	
III (undefined)	6 (2.7%)	3 (1.3%)	
IIB	0 (0%)	1 (0.4%)	0.026
N. metastatic sites			
<3	114 (52%)	83 (37%)	
≥3	100 (46%)	121 (54%)	
Unknown	5 (2%)	20 (9%)	0.01
Brain metastases			

Absent	164 (75%)	161 (72%)	
Present	55 (25%)	73 (28%)	0.5
Liver metastases			
Absent	197 (90%)	194 (87%)	
Present	22 (10%)	30 (13%)	0.3
Tumor burden≥64mm			
No	53 (24%)	37 (16%)	
Yes	104 (47%)	80 (35%)	
Unknown	62 (28%)	107 (48%)	0.7
Tumor burden>102mm			
No	100 (46%)	76 (34%)	
Yes	57 (26%)	41 (18%)	
Unknown	62 (28%)	107 (48%)	0.8
LDH-NLR			
Good	25 (11%)	24 (11%)	
Intermediate	94 (43%)	89 (40%)	
Poor	48 (22%)	58 (26%)	
Unknown	52 (24%)	53 (23%)	0.6
KRAS			
Wild type	111 (51%)	113 (50%)	
Mutant	86 (39%)	71 (32%)	
Unknown	22 (10%)	40 (18%)	0.3
Corticosteroids≥10mg			
Yes	48 (22%)	55 (24%)	
No	157 (72%)	142 (63%)	
Unknown	14 (6%)	27 (12%)	0.3
Proton pump inhibitors			
Yes	82 (37%)	84 (37%)	
No	118 (54%)	109 (49%)	
Unknown	19 (9%)	31 (14%)	0.6
Treatment			
Pembro	219 (100%)	-	
Pembro + carbo + pem	-	159 (71%)	
Pembro + cis + pem	-	27 (12%)	
Pembro + carbo + tax	-	29 (13%)	
Pembro + carbo + abr	-	8 (3.5%)	

Pembro + carbo + gem	-	1 (0.5%)	

Table 1 Baseline characteristics of included patients

With a median follow up (FUP) of 40.4 [95% confidence interval (95%CI) 38.5 - 46.9] months in the pembrolizumab group and 30.4 (95%CI 27.2-32.2) months in the combination group, median OS was 22.2 (95%CI 16.5 – 30.2) and 15.5 (95% CI 12.4 – 20.7) months, respectively . PFS was 6.9 (95% CI 5.2 – 91) in the pembrolizumab and 9.1 (95%CI 7.7 – 10.9) in the combination group.

3.1 *Prognostic factors in the overall population*

We investigated the role of clinical (age, gender, PS ECOG, number of metastatic sites, presence of brain and/or liver metastases, concomitant treatment with corticosteroids<10mg and/or proton pump inhibitors), pathological/molecular (histology, *KRAS* status), radiological (tumor burden at diagnosis≥64 mm and <102mm) features, and the treatment (pembrolizumab/pembrolizumab-chemotherapy) as prognostic factors in the general population treated with first line pembrolizumab as single agent or in combination with chemotherapy.

In the multivariable analysis, older age (p=0.03), PS ECOG≥2 (p<0.001), the presence of *KRAS* mutations (p=0.02), LDH-NLR poor before any treatment start (p=0.03), a tumor burden>102 mm (p=0.02), concomitant treatment with corticosteroids (p=0.02) and proton pump inhibitors (p=0.01) were independent, negative prognostic factors. The analysis was conducted through a stratification per treating facility.

3.2 *Overall survival*

Overall survival results in pre-defined subgroups are gathered in Table 2.

Younger (p=0.007) and male patients (p=0.01), patients with PS ECOG 0-1 at diagnosis of metastatic disease (p=0.04), with an adenocarcinoma (p=0.02) and not harboring *KRAS* mutations (p=0.03) seemed to benefit from pembrolizumab monotherapy compared to pembrolizumab-chemotherapy. The interaction test confirmed an interaction between the treatment and age (p=0.04), the treatment and the PS ECOG (p<0.001), the treatment and tumor histology (p=0.007 for squamous and p=0.01 for other, non-adenocarcinoma histology).

In the small subgroup of patients with LDH-NLR “good” (n=48) median OS to first line pembrolizumab (n=24) was 41.8 months; median OS was not reached (NR) with pembrolizumab-chemotherapy (n=24), p=0.33.

In patients with liver metastases at baseline (n=52), median OS was numerically higher with pembrolizumab-chemotherapy as first line treatment [10.4 vs 3.3 months, HR 1.19 (95%CI 0.64 - 2.21), *p*=0.56]. Female patients achieved a slightly higher OS with combination therapy [20.4 vs 24.4 months, HR 1.06 (95%CI 0.69 – 1.61), *p*=0.78] (Table 2).

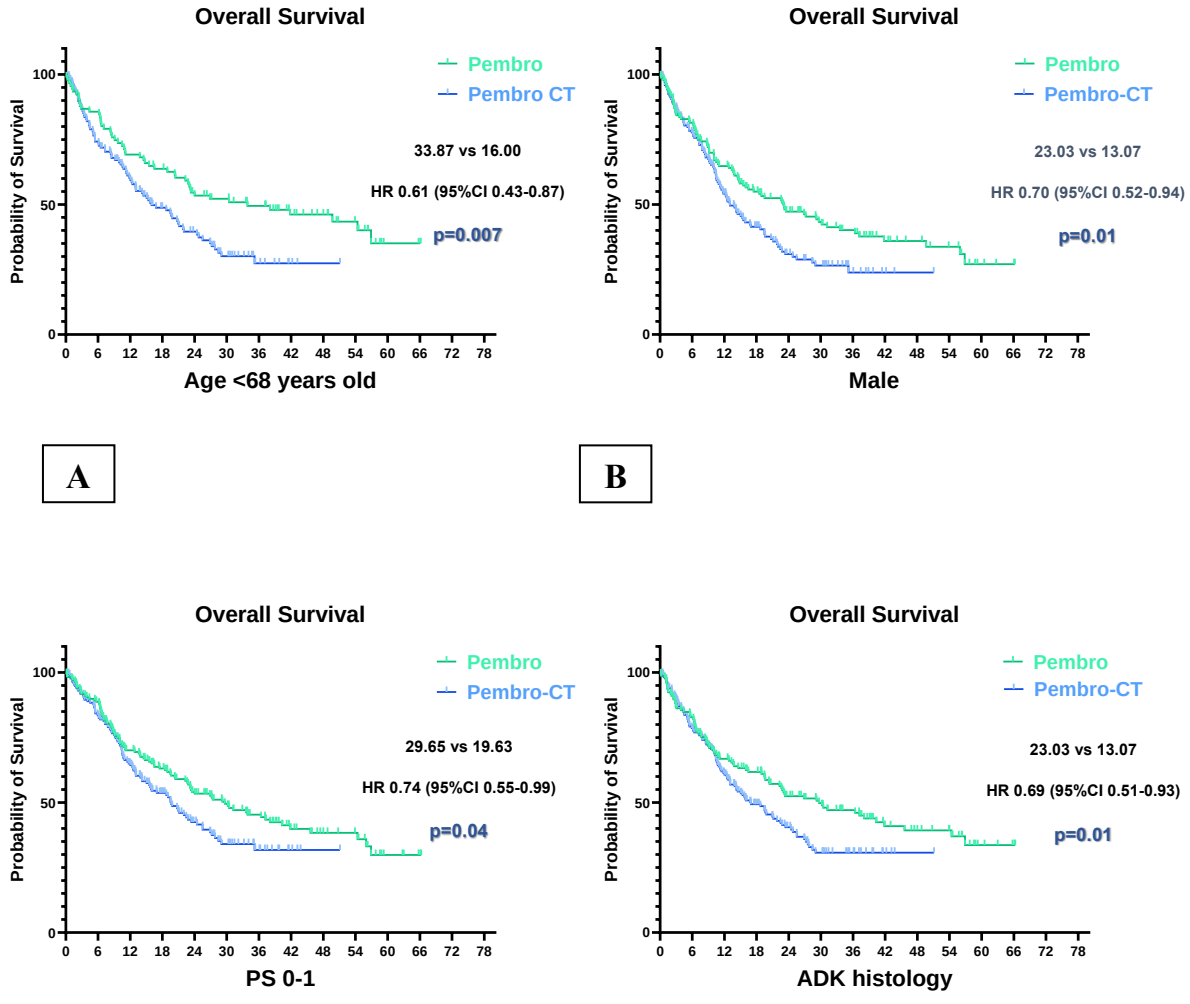
Data regarding subsequent lines were available for 138 patients treated with first line pembrolizumab, out of 169 who experienced disease progression (82%). 45 out of 138 (33%) received platinum-based therapy as subsequent treatment line. Among male patients treated with pembrolizumab alone, cross-over rate to platinum-based chemotherapy was 33%; among patients younger than 68 years old, it was 44%. For patients with PS ECOG 0-1 at diagnosis, treated with first line pembrolizumab, cross-over rate to platinum-based chemotherapy in second line was 36%, whereas for patients with adenocarcinoma histology it was 42%. Among patients whose tumors harbored *KRAS* mutations, cross-over rate was 37%.

	Pembro N	Pembro-CT N	Pembro vs Pembro-CT (months)	HR (95%CI)	*p*
Age					
<68	91	130	33.8 *vs* 16.0	0.62 (0.44-0.88)	0.007*
≥68	128	94	16.4 *vs* 13.9	0.97 (0.69-1.37)	0.9
Gender					
Male	141	151	23.0 *vs* 13.0	0.70 (0.52-0.94)	0.01*
Female	78	73	20.4 *vs* 24.4	1.06 (0.69-1.61)	0.78
Never smokers	14	23	22.8 *vs* 15.3	0.71 (0.33-1.52)	0.37
PS ECOG					
0-1	169	176	29.6 *vs* 19.6	0.75 (0.56-1.00)	0.04*
≥2	50	48	4.6 *vs* 6.9	1.03 (0.66-1.60)	0.88
Histology					
Adenocarcinoma	146	182	29.6 *vs* 16.9	0.72 (0.54-0.96)	0.02*
Non-adenocarcinoma	73	42	14.6 *vs* 9.2	0.85 (0.53-1.35)	0.48
N. metastatic sites ≥3	100	121	13.8 *vs* 12.0	0.83 (0.60-1.14)	0.25
Brain metastases	55	63	20.3 *vs* 15.0	0.82 (0.51-1.82)	0.42
Liver metastases	22	30	3.3 *vs* 10.4	1.19 (0.64-2.21)	0.56
Tumor burden≥64mm	104	80	14.6 *vs* 13.9	0.82 (0.57-1.18)	0.26
Tumor burden>102mm	57	41	14.5 *vs* 10.5	0.67 (0.41-1.10)	0.09

LDH-NLR					
Good	25	24	41.8 *vs* NR	0.66 (0.28-1.57)	0.33
Intermediate – poor	142	147	14.4 *vs* 14.2	0.93 (0.70-1.24)	0.64
KRAS					
Mutant	86	71	20.3 *vs* 18.7	0.84 (0.56-1.27)	0.41
Wild type	111	113	26.8 *vs* 14.2	0.70 (0.50-0.98)	0.03*
Corticosteroids≥10mg	48	55	6.8 *vs* 8.2	1.14 (0.74-1.77)	0.52
Proton pump inhibitors	82	84	12.8 *vs* 10.2	0.90 (0.63-1.29)	0.59

Table 2 Overall survival in pre-specified subgroups

** statistically significant results*



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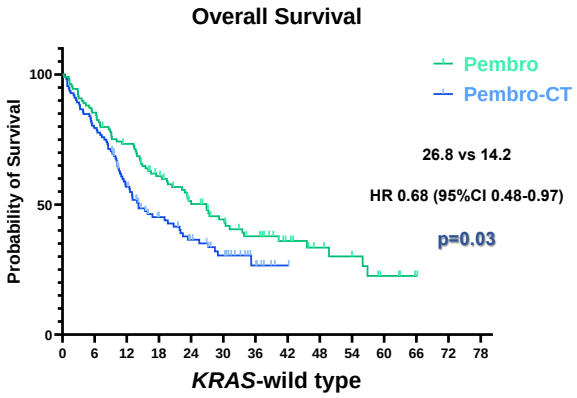

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Figure 2. Overall survival in a) age<68 years old (median) b) male patients c) PS ECOG 0-1 d) adenocarcinoma histology e) KRAS-wild type

3.3 *Progression-free survival*

PFS results in pre-specified subgroups are gathered in Table 3.

PFS was significantly longer with combination therapy in patients with NLR-LDH intermediate or poor (p=0.04) and numerically doubled (22.0 vs 11.2 months) with pembrolizumab single agent in NLR-LDH good [HR 0.62 (95%CI 0.31 - 1.26), p=0.18]. On the contrary, some subgroups (i.e., female patients, patients with liver metastases, with at least three metastatic sites and treated with corticosteroids≥10mg) had numerically longer median PFS with combination therapy, although statistical significance is not reached (Table 3).

To note, PFS to pembrolizumab-chemotherapy was three time longer than to pembrolizumab alone (6.2 vs 2.2 months) in patients with PS ECOG≥2. The determinant for PS ECOG≥2 was cancer-burden and cancer-related symptoms in 42 (93%) patients treated with pembrolizumab-chemotherapy and 32 (67%) patients treated with pembrolizumab alone, thus constituting the most frequent cause of "frailty". Concomitant comorbidities were the determinant of PS ECOG≥2 in 3 (7%) patients treated with combination therapy

and 10 (21%) patients treated with pembrolizumab alone. In the remaining cases (six patients treated with pembrolizumab, 12%), the determinant was unknown.

	Pembro N	Pembro-CT N	Pembro vs Pembro-CT (months)	HR (95%CI)	*P*
Age					
<68	91	130	11.5 *vs* 10.5	0.84 (0.62-1.15)	0.29
≥68	128	94	6.3 *vs* 7.6	0.99 (0.74-1.32)	0.95
Gender					
Male	141	151	7.1 *vs* 8.4	0.87 (0.67-1.12)	0.28
Female	78	73	5.1 *vs* 9.7	1.17 (0.82-1.67)	0.37
Never smokers	14	23	10.5 *vs* 9.4	0.78 (0.39-1.54)	0.47
PS ECOG					
0-1	169	176	8.1 *vs* 9.7	0.90 (0.70-1.14)	0.39
≥2	50	48	2.2 *vs* 6.2	1.20 (0.80-1.90)	0.31
Histology					
Adenocarcinoma	146	182	7.1 *vs* 9.8	0.89 (0.69-1.14)	0.35
Non-adenocarcinoma	73	42	5.0 *vs* 7.1	1.06 (0.70-1.60)	0.77
N. metastatic sites ≥3	100	121	4.2 *vs* 8.3	1.09 (0.82-1.46)	0.51
Brain metastases	55	63	7.1 *vs* 9.9	0.88 (0.59-1.32)	0.55
Liver metastases	22	30	2.6 *vs* 8.3	1.19 (0.65-2.19)	0.53
Tumor burden≥64mm	104	80	6.9 *vs* 8.2	0.95 (0.68-1.31)	0.76
Tumor burden>102mm	57	41	7.0 *vs* 7.0	0.81 (0.52-1.28)	0.36
LDH-NLR					
Good	25	24	22.0 *vs* 11.2	0.62 (0.31-1.26)	0.18
Intermediate – poor	142	147	4.9 *vs* 9.4	1.29 (1.00-1.66)	0.04*
KRAS					
Mutant	86	71	6.1 *vs* 10.7	1.02 (0.72-1.45)	0.88
Wild type	111	113	8.1 *vs* 9.4	0.95 (0.71-1.28)	0.77
Corticosteroids≥10mg	48	55	2.1 *vs* 5.4	1.39 (0.91-2.10)	0.10
Proton pump inhibitors	82	84	4.6 *vs* 5.6	1.07 (0.77-1.49)	0.65

Table 3 Progression-free survival in pre-specified subgroups

**statistically significant results*

4.0 Discussion

PD-L1 expression level is the only discriminant for choosing between pembrolizumab alone or in combination with chemotherapy for advanced, previously untreated, non-oncogene addicted NSCLC(1). Patients whose tumors express PD-L1 on at least 50% of tumor cells might receive pembrolizumab as single agent(5), whereas the combination of pembrolizumab and chemotherapy has shown survival benefit over chemotherapy regardless of PD-L1 expression(8–11). Given the limitations of PD-L1 as predictive factor, the identification of clinical, biological, radiological factors that might guide the physician in the choose of the best treatment for the most suitable patient is still an unmet need in this setting. Here, we present the results of a retrospective analysis conducted on real world population treated at three health facilities in Europe, for a total of more than 400 patients who received pembrolizumab or pembrolizumab-chemotherapy.

At a median FUP of 40.4 and 30.4 months in the pembrolizumab and pembrolizumab-chemotherapy group, respectively, median OS were 22.2 (95%CI 16.5 – 30.2) in the pembrolizumab group, thus slightly shorter than reported in the pivotal trial (26.3 months)(5); 15.5 (95% CI 12.4 – 20.7) months in the combination group, thus slightly shorter than showed in the five-years follow up of registering clinical trials (22.0 in non-squamous and 17.2 months in squamous histology)(10,11).

This real-world population showed some imbalances between the two treatments arms, reflecting how physicians tend to favor one treatment over another in clinical practice. Older patients tended to be treated with pembrolizumab alone (p=0.03), and the same was seen in patients with non-adenocarcinoma histology (p=0.002). Notably, patients with squamous histology tended to be older (p=0.0000003) and to present with a PS ECOG≥2 at diagnosis (p=0.004). Thus, as expected, "frail" patients are more frequently treated with immunotherapy alone. This is applicable only to patients whose tumors express PD-L1 on at least 50% of tumor cells; otherwise, these patients might be unfit for a platinum-based combination and be candidate to single agent chemotherapy (a sub-population that it is not represented in this study). Moreover, as aforementioned, prescribing limitations in Italy reserve pembrolizumab-chemotherapy for PD-L1 expression levels<50%. As a consequence, if a patient is fit for platinum, treatment strategy (pembrolizumab or combination therapy) is based solely on PD-L1, with no possibility of choosing according to clinical features; but if a patient is unfit for platinum, pembrolizumab as single agent might be proposed only if PD-L1 is ≥50%, whereas with PD-L<50% the patient might be eligible to single agent chemotherapy only.

On the other hand, patients with at least three metastatic sites are more frequently treated with combination therapy (p=0.01), reflecting a physician's tendency to consider it a more aggressive disease, at least among

patients within the French cohort, for whom pembrolizumab-chemotherapy could be employed irrespective of PD-L1 expression. Indeed, those patients (n=29) who, despite a PD-L1≥50%, received pembrolizumab-chemotherapy, were likely to have at least three metastatic sites at presentation (76%); half of them had brain metastases at diagnosis, and they were younger compared to the general population (median age 63 *vs* 68 years old).

Older age (p=0.03), PS ECOG≥2 (p<0.001), *KRAS* mutations (p=0.02), LDH-NLR poor (p=0.03), a tumor burden>102 mm (p=0.02), concomitant treatment with corticosteroids (p=0.02) and proton pump inhibitors (p=0.01) at the time of treatment start were independent, negative prognostic factors in the overall population (patients treated with either pembrolizumab or pembrolizumab-chemotherapy). Age and PS ECOG≥2 are known prognostic factors in advanced NSCLC(22); baseline sum of largest diameters>102 and LDH over the upper limit of normal reflect a larger tumor burden and are known as negative prognostic factors and predictors of worse outcomes with immunotherapy(29,43). Here, we combined LDH and NLR in a three-category score, providing a prognostic stratification irrespective of the addition of chemotherapy to first line pembrolizumab. Concomitant treatments with corticosteroids and/or proton pump inhibitors are known to impair outcomes with immunotherapy. In this real-world study, they arise as negative prognostic factors in the overall populations (patients treated with pembrolizumab with or without chemotherapy). Due to the small sample of patients receiving baseline corticosteroids (n=48, 22% and n=55, 24% in pembrolizumab and pembrolizumab-chemotherapy group respectively) the reasons that led to the treatment (cancer related symptoms or pre-existing conditions) were not considered in the analysis. Moreover, proton pump inhibitors are often prescribed together with corticosteroids, for gastro protection purposes: in our cohort, 78 out of 166 (47%) patients receiving proton pump inhibitors received a concomitant treatment with corticosteroids≥10 mg of prednisone equivalent.

Interestingly, the presence of a *KRAS* mutation appeared to be an independent, negative prognostic factor, although no data concerning co-occurring alterations in *STK11* and *KEAP1* were available. Mutations in *STK11* and *KEAP1* occur in 15% of adenocarcinomas of the lung and 20% of NSCLC, respectively, and, as aforementioned, they are negative prognostic factors for advanced NSCLC(58). Indeed, while they had already been recognized as predictors of worse response and survival outcomes to single-agent immunotherapy(55,57), recent evidence show that the presence of mutations in oncosuppressors (i.e. *STK11, KEAP1, SMARCA4*) impairs outcomes to immunotherapy even when combined with chemotherapy(58). In our population, a baseline molecular profile assessed through a panel including *STK11, KEAP1, TP53, SMARCA4* was available only in approximately one third (n=149, 34%) of patients. Thus, the co-occurring molecular alterations were not considered in the analysis.

Pembrolizumab as single agent provided an OS benefit over the combination with chemotherapy in <68 years old (p=0.007), male patients (p=0.01), patients with PS ECOG 0-1(p=0.04), with an adenocarcinoma (p=0.02) and not harboring *KRAS* mutations (p=0.03). The interaction between the treatment and the

clinical feature was confirmed for age (p=0.04), PS ECOG (p<0.001), and tumor histology (p=0.007 for squamous and p=0.01 for other, non-adenocarcinoma histology). Then, we analyzed cross-over rates to platinum-based chemotherapy at progression to investigate whether patients with more favorable clinical features might be more likely to be eligible for platinum in subsequent lines. While cross-over rate was 33% in the overall population treated with pembrolizumab, it was slightly higher in younger patients (44%) and adenocarcinoma histology (42%), but similar in the other cases.

Overall, these results suggest that some "good prognosis" patients might benefit from single agent immunotherapy as first line treatment. On the contrary, the addition of chemotherapy might be useful to counteract the negative impact of some baseline clinical/biological features. Patients with intermediate/poor LDH-NLR obtained a significantly longer PFS with combination therapy compared to pembrolizumab alone (9.4 *vs* 4.9 months, p=0.04). Although not significant, PFS to pembrolizumab-chemotherapy was three time longer than to pembrolizumab alone (6.2 *vs* 2.2 months) in patients with PS ECOG≥2. To note, the most common determinant for PS ECOG≥2 was cancer-burden related symptoms (93% in patients treated with combination and 67% of cases in patients treated with pembrolizumab). In line with this result, patients with at least three metastatic sites at treatment start had a numerically doubled PFS to combination therapy compared to pembrolizumab alone, and those with liver metastases at baseline had a three-time longer PFS with pembrolizumab-chemotherapy (Table 3).

This study has certain limitations that need to be acknowledged. Firstly, the retrospective nature of this research made it impossible to retrieve the baseline radiological evaluations for all patients and assess the sum of the longest diameters according to RECIST 1.1. Therefore, data regarding the radiologically assessed tumor burden are missing in almost one third of patients treated with pembrolizumab and almost the half of patients treated with pembrolizumab-chemotherapy. Similarly, it was not possible to analyze the impact of concomitant mutations in *STK11/KEAP1/TP53* in the *KRAS*-mutant subgroup, due to a difference in techniques and panels used in different facilities. Moreover, while in Italy pembrolizumab-chemotherapy is reserved for PD-L1 expressions<50%, this strategy is reimbursed for any PD-L1 expression in France. Only 29 patients with PD-L1≥50% were treated with immune-chemotherapy, hence a comparison between the two treatments in the high PD-L1 subgroup was not possible due to small sample size.

5.0 Conclusions

Patients younger than 68 years old, with PS ECOG 0-1 at diagnosis, and with tumors of adenocarcinoma histology achieved longer OS with pembrolizumab compared to pembrolizumab plus platinum-based chemotherapy as first line treatment for advanced NSCLC. To note, pembrolizumab as single agent is approved (and it was administered) only for PD-L1 TPS ≥50%.

On the contrary, the addition of platinum-based chemotherapy might mitigate the negative effect of a large tumor burden, reflected by the presence of at least three metastatic sites at the diagnosis, the presence of liver metastases, and PS ECOG impaired by tumor-related symptoms.

NLR-LDH categorization in "good", "intermediate", "poor" arises as new, independent, negative prognostic factor, regardless the addition of chemotherapy to first line pembrolizumab.

Results from prospective studies comparing pembrolizumab and pembrolizumab-chemotherapy, especially when PD-L1≥50%, are extremely warranted.

List of abbreviations

Abr: nab-paclitaxel; ADK: adenocarcinoma; carbo: carboplatin; cis: cisplatin; CT: chemotherapy; EAP: expanded access program; EMA: European Medicines Agency; FDA: Food and Drug Administration; FFPE: formalin-fixed/paraffin-embedded; FUP: follow up; gem: gemcitabine; HR: hazard ratio; IHC: immunohistochemistry; IQR: interquartile range; KEAP1: Kelch-like ECH-associated protein 1; LDH: lactate dehydrogenase; MTV: metabolic tumor volume; N: number; NLR: neutrophil-to-lymphocyte ratio; NR: not reached; OS: overall survival; PD-(L)1: programmed-death (ligand) 1; Pembro: pembrolizumab; Pembro-CT: pembrolizumab-chemotherapy; PFS: progression free survival; PS: performance status; SITC: Society for the Immunotherapy of Cancer; STK11: Serine/threonine kinase 11; TAM: tumor-associated macrophage; TPS: tumor proportional score; TP53: tumor protein 53; Treg: regulatory T cell; tax: paclitaxel; 95%CI: 95% confidence interval.

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