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Real-world evidence: analysis of oncological drug expenditure and consumption, assessment of prescriptive appropriateness for clinical government of cancer immunotherapy

Evidenze dal mondo reale: Analisi dei consumi e dei comportamenti prescrittivi di farmaci immunoterapici oncologici per il governo clinico

Relatore: Chiar.mo Prof. Nicola Realdon

Co-relatore: Dott. Enrico Gori

Laureando: Ellen Cossu

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ABSTRACT

Background Lung cancer is one of the top 10 causes of death globally and as well as in Italy, as in the rest of the world, it is the most common cancer in terms of incidence.

Immune checkpoint inhibitors (ICIs) have become the gold standard of care in the setting of lung cancer in patients without targetable genetic changes. Since 2015, immunotherapy changed the paradigm of Non-Small Cell Lung Cancer (NSCLC) treatment in different settings and has contributed to improve the quality of life of these patients. The anti-PD-1 pembrolizumab and nivolumab, anti-PD-L1 inhibitor atezolizumab, and the anti-CTLA4 inhibitor ipilimumab are the checkpoint inhibitors used in advanced NSCLC. PD1/PDL1 inhibitors have a particular mechanism of action, they unblock the T cells immune suppression which results in T cells multiplication and permeation into the tumor microenvironment inducing an immune response. These treatments are defined as innovative due to their mechanism of action, therapeutic need and clinical benefit. Being first-in-class drugs, they have a high cost which greatly affects the pharmaceutical expenditure of the Italian National Health System (NHS).

In a limited-resource system, such as the NHS, the evaluation of drug use must be considered a fundamental element for a thoughtful allocation of resources and to ensure that all citizens have access to appropriate treatment. The introduction of new high-cost treatments on the market, as for lung cancer, has called for the development of a tool to evaluate in both clinical and economic terms the prescriptive appropriateness of these therapies, highlighting areas of over- and under-utilization, and defining the correct place in therapy.

Aim of the study Analysing the costs and consumption of these drugs, it was necessary to describe, in the real clinical practice context of NSCLC, the treatment modalities and clinical outcomes observed with the antibodies. Therefore, the study aimed to compare the clinical outcome of ULSS 3 Serenissima patients with the pivotal studies of the ICIs, by checking the presence of the eligibility conditions that allows a prescription to be defined as appropriate.

Materials and methods The project conducted in ULSS 3 Serenissima is an observational, retrospective, single-centre study performed using real-world data from administrative databases, the onco-haematological therapy management systems and the AIFA Monitoring Registers. The materials used coincide with data obtained from the ULSS 3 population of 625,189 patients. The data on patients, the four oncology departments, and drugs administered enabled the writing of this paper. The study is divided into three sub-studies analysing the appropriateness and clinical efficacy of the following drugs: Pembrolizumab, Nivolumab, Atezolizumab in the hospital districts of Chioggia, Mestre, Venice, Dolo and Mirano. The

clinical outcomes of the study used to compare real-life treatment performance with that reported in the pivotal studies were: PFS = median progression free survival, OS = median overall survival, 6 months survival rate, 6 months progression rate, ORR = objective response rate, DOR = duration of response. On the other hand, the analysis of prescriptive appropriateness was conducted by comparing eligibility criteria entered in the AIFA monitoring registries with those noted from pathology anatomy reports, and the management system for the setting up of haemato-oncology therapies.

Results In 2021 the sum of spending on these antibodies amounted to 3.050.629,12 euros which represents 2.32% of total annual pharmaceutical spending. Pembrolizumab median overall survival (OS) was 11.2 months (95% confidence interval [CI], 6,87-33,1). The ULSS 3 median progression free survival (PFS) was 4.5 months (95% confidence interval [CI], 3,5 - 9,1). Nivolumab median overall survival (OS) was 11,4 months (95% confidence interval [CI], 7,27 – 23,4). The ULSS 3 median progression free survival (PFS) was 3.91 months (95% confidence interval [CI], 22,66 - 12,7). Atezolizumab median overall survival (OS) was 9,75 months (95% confidence interval [CI], 4,96 – 18). The ULSS 3 median progression free survival (PFS) was 3,5 months (95% confidence interval [CI], 22,66 - 12,7). No significant difference was found between nivolumab and atezolizumab treatment. There were a few cases of patients presenting disagreed data with pathology reports or presenting no reports from 2016 to 2022. Many more were patients with unconfirmable eligibility criteria because they were not detected in the reports. No significant difference in survival values (OS and PFS) was found in patients with correct and comparable eligibility criteria when compared patients with discordant or unconfirmable eligibility criteria.

Conclusion First-line monotherapy with pembrolizumab seems less effective in the real population than in pivotal clinical trials. Atezolizumab and nivolumab have similar efficacy to Randomized Clinical Trial (RCTs). PS ECOG, histologic characterization, and PD-L1 were not identified as predictors of response. Equal efficacy was demonstrated between atezolizumab and nivolumab. Although it has not been statistically proven that the choice of therapy based on incorrect eligibility criteria led to adverse therapeutic outcomes (progression and death), it remains of paramount importance to choose the therapy according to the eligibility criteria indicated in guidelines or monitoring registries. The results show a good level of prescriptive appropriateness in ULSS 3 for the drugs analysed and a few cases of overuse.

RIASSUNTO

Presupposti dello studio Il tumore al polmone è una tra le prime dieci cause di morte sia in Italia che a livello globale e costituisce il tumore più comune in termini di incidenza.

Gli inibitori del checkpoint immunitario (ICIs) sono diventati il gold standard di cura per il carcinoma polmonare avanzato e metastatico in pazienti senza alterazioni genetiche target.

Dal 2015, l'immunoterapia ha cambiato il paradigma del trattamento del *Non Small Cell Lung Cancer* (NSCLC) in diversi contesti e ha contribuito a migliorare la qualità di vita dei pazienti.

Gli inibitori anti-PD-1 pembrolizumab e nivolumab, l'inibitore anti-PD-L1 atezolizumab e l'inibitore anti-CTLA4 ipilimumab sono gli inibitori del checkpoint utilizzati nel NSCLC avanzato. Gli inibitori di PD1/PDL1 hanno un meccanismo d'azione particolare: interrompono l'immunosoppressione delle cellule T, con conseguente moltiplicazione e permeazione delle stesse nel microambiente tumorale e inducono una risposta da parte del sistema immunitario. Per il loro particolare meccanismo d'azione, la necessità terapeutica e il beneficio clinico fornito, questi trattamenti sono definiti innovativi. Essendo farmaci *first in class*, hanno un costo elevato, che incide notevolmente sulla spesa farmaceutica del Sistema Sanitario Nazionale (SSN). In un sistema a risorse limitate, come il SSN italiano, la valutazione dell'uso dei farmaci deve essere considerata un elemento fondamentale per una ponderata allocazione delle risorse e per garantire a tutti i cittadini l'accesso a cure appropriate. L'introduzione sul mercato di nuovi trattamenti ad alto costo, come per il tumore al polmone, ha richiesto lo sviluppo di uno strumento per valutare in termini sia clinici che economici l'appropriatezza prescrittiva di queste terapie, evidenziando le aree di sovra e sottoutilizzo e definendo la corretta collocazione della terapia.

Obiettivo dello studio Analizzando i costi e i consumi di questi farmaci, è stato necessario descrivere nel reale contesto della pratica clinica del NSCLC, le modalità di trattamento e gli esiti clinici osservati con gli anticorpi. Lo scopo dello studio è stato quindi quello di confrontare l'esito clinico dei pazienti dell'ULSS 3 Serenissima con gli studi registrativi degli ICIs, verificando la presenza delle condizioni di eleggibilità che consentono di definire appropriata la prescrizione.

Materiali e metodi Il progetto condotto nell'ULSS 3 Serenissima è uno studio osservazionale, retrospettivo, monocentrico, realizzato utilizzando dati reali provenienti da database amministrativi, dai gestionali per l'allestimento delle terapie onco-ematologiche e dai registri di monitoraggio AIFA.

I materiali utilizzati coincidono con i dati ottenuti dalla popolazione ULSS 3 di 625.189 pazienti. I dati sui pazienti, sui quattro reparti oncologici e sui farmaci somministrati hanno permesso la stesura di questo elaborato.

Lo studio è suddiviso in tre sotto-studi che analizzano l'appropriatezza e l'efficacia clinica dei seguenti farmaci: pembrolizumab, nivolumab, atezolizumab nei distretti ospedalieri di Chioggia, Mestre, Venezia, Dolo e Mirano.

Conclusioni La monoterapia di prima linea con pembrolizumab sembra meno efficace nella popolazione reale rispetto agli studi clinici registrativi. Atezolizumab e nivolumab hanno un'efficacia simile a quella degli studi clinici randomizzati. Il PS ECOG, la caratterizzazione istologica e il PD-L1 non sono stati identificati come fattori predittivi di risposta. È stata dimostrata la stessa efficacia tra atezolizumab e nivolumab. Nonostante non sia stata dimostrata una differenza di efficacia significativa tra pazienti con criteri di eleggibilità non confermabili e confermati, rimane di fondamentale importanza che la scelta della terapia avvenga in base ai criteri di eleggibilità indicati dalle linee guida o dai registri di monitoraggio. I risultati mostrano un buon livello di appropriatezza prescrittiva per i farmaci analizzati e pochi casi di sovra utilizzo nell'ULSS 3.

1. INTRODUCTION

In the Italian context, lung cancer is one of seven tumors with the worst prognosis¹, complicated therapeutic approaches and a national annual expenditure of about €2.5 billion².

The management of this disease has evolved extremely during the past decades, particularly in the pharmacological treatments, which are mainly based on chemotherapy, target therapy and immunotherapy. The introduction of tumor agnostic therapy, based on cancer's genetic and molecular features without regard to cancer type or where cancer started in the body, has changed the landscape of lung cancer treatment but also the impact on government spending.

In the following chapter, it is necessary to introduce some general aspects in order to better understand the multiple treatment modalities of this disease.

1.1 Epidemiology of lung cancer

To understand the severity of this disease it is useful to report some common indicators in cancer statistics: incidence, mortality and survival.

According to the WHO's 2019 data, lung cancer is the sixth world cause of death³, and it can be defined as a global problem and public health issue.

Lung cancer cases and deaths are climbing in developing countries in conjunction with tobacco smoking⁴. As well as in high-income countries, where smoking has decreased⁵, disease understanding, treatment options and outcomes for lung cancer are improving, survival continues to be low.

In Italy, in 2017, there were 33.904 registered deaths from lung cancer, which represented the leading cause of cancer death in men and the second leading cause of cancer death (after breast cancer) in women⁶.

Veneto Tumor Registry discloses that the incidence rate varies according to age and sex, but for both men and women in the 50-69 and +70 age groups, lung cancer remains among the four most frequent neoplasms⁷. Always in the Veneto region, over the last 20 years, the incidence of lung cancer has decreased progressively in males, particularly in 2000. In women, however, there was a slight increase in incidence from 1990 to 2009. Despite these small changes about incidence, the five-year relative survival rate from the diagnosis of subjects with lung cancer, diagnosed in the four years 2006-2009, was 12.3% in males and 15.7% in females⁸. Although survival is greater for localized stage diagnoses and before the age of 45, these cases represent only 15% and 27% respectively⁹, therefore lung cancer can be defined as a tumor with a poor prognosis.

The main reason for the poor prognosis of this tumor is the lack of early signs and symptoms, which usually determines a diagnosis at an advanced stage and therefore also limited effectiveness of the available therapeutic tools.

1.2 Classification of lung cancer

The identification of the cell morphology, the histology, the presence of driver mutation and the stage of the cancer are the key to a proper prescription. Especially with the introduction of personalized treatments, histologic classification and biomarker information play an increasingly pivotal role in the diagnosis and management of lung cancer.

Lung cancer is a very heterogeneous pathology, both cellular and histological level. 2021 WHO classification reports several types of tumors through three principles: morphology first supported by immunohistochemistry, and then molecular techniques¹⁰.

The main subdivision is about the tissue in which the tumor originates, although 95% of all cancers affecting these organs derives from the epithelial structures surrounding the lung. The remaining 5% includes neuroendocrine neoplasm (in this case we talk about lung carcinoid), cancers of ectopic tissues, mesenchymal and haematolymphoid tumors (in this case it is pulmonary lymphoma)¹¹.

According to cell morphology, lung cancer is traditionally divided into two main groups: small-cell lung carcinoma (SCLC, 13% of the cases) and non-small-cell lung carcinoma (NSCLC, 83 % of the cases)¹². Classifying the tumor mass between these two types of cancer has an important impact on defining prognosis and also on therapeutic decisions. SCLCs are treated non-surgically, usually with chemotherapy, alone or combined with radiation and in advanced SCLC might be treated with or without immunotherapy; whereas NSCLCs are managed by a combination of surgery and adjuvant therapy¹³. These two groups are then divided into other subtypes as can be seen from the figure 1.2.

Looking at the complexity and at the greater use of immune checkpoint inhibitors in the treatment of NSCLC, the SCLC will not be too deepened, to shift attention to the sub-types of non-small cell lung cancer

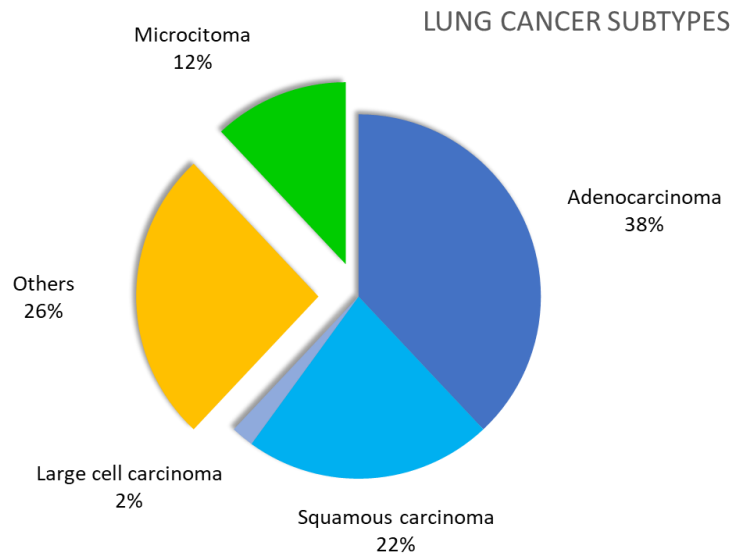


Figure 1.2 Representation of the histological classification of lung cancer according to AIRTUM 2014 data.

1.2.1 Small Cell Carcinoma (SCLC)

Small cell lung cancer is a much less common form of lung cancer, but it is a tumor particularly aggressive that is often diagnosed in the advanced or metastatic stage. Indeed most patients relapse within the first 2 years after treatment and the 2-year survival rate is less than 10% in metastatic patients¹⁴. Small cell lung cancer has rapid metastatic dissemination with a high proliferation index, it is very sensitive to chemotherapy despite the results are not durable.

SCLC is classified as neuroendocrine cancer, and it is divided into 2 subtypes: pure SCLC and combined SCLC, containing 10% of large cell carcinoma component ¹⁵.

It is comprised of small-medium sized cells almost represented by the nucleus and by a thin cytoplasmic layer. Immunohistochemistry (IHC) can be very helpful in excluding other morphological features because the cytoplasm of its cells is immunopositive for neuroendocrine markers, such as CD56, chromogranin and synaptophysin.

SCLC is commonly centrally located in the major airway and it is generally easily accessible, for this reason the most common method to obtain a pathological diagnosis is a biopsy taken either by bronchial endoscopy, or from a lymph node (by bronchial or transesophageal endoscopy, supraclavicular node biopsy or mediastinoscopy), or from a metastasis (subcutaneous, hepatic, bone, ...).

The correlation with smoking has proven to be very significant in SCLC, as in squamous cell carcinoma, where squamous metaplasia is the typical morphological modification of the respiratory epithelium associated with smoking⁵.

1.2.2 Non-Small Cell Carcinoma (NSCLC)

Non-small cell lung cancer, also known as oat cell cancer, is the most common type of lung cancer and it includes three main subtypes: adenocarcinomas (AC), squamous cell carcinomas (SSC) and large cell carcinomas (LCC).

The availability of targeted therapies led to the need to identify a precise subtype of NSCLC.

Differentiating between adenocarcinoma (AC) and squamous cell carcinoma (SCC) had therapeutic relevance, because new therapies have been developed that have different therapeutic or adverse effects depending on the histologic type.

1.2.2.1 Adenocarcinoma

Adenocarcinoma is the most common type of lung cancer, and it is a malignant epithelial tumor with glandular differentiation or mucin production, located on the outer part of the lungs. It most affects women, non-smokers, and young people.

Lung adenocarcinomas frequently show mucin production, which is detectable by mucin staining like pneumocyte marker expression like napsin A or thyroid transcription factor 1 (TTF1)¹⁶.

Adenocarcinoma is the most prevalent histological form in non-smokers, unlike squamous cell carcinoma is more frequently linked to smoking¹⁷.

Adenocarcinoma can present diverse histological patterns, which can be located in the same tumor including lepidic, acinar, papillary, micropapillary, and solid patterns. While the lepidic pattern is associated with a favorable prognosis, micropapillary and solid patterns are associated with more aggressive behavior¹⁸.

1.2.2.2 Squamous cell carcinoma

Squamous cell carcinoma (SCC), although many times at diagnosis is already expanded in the periphery, usually occurs in the origin of the tracheobronchial tree, in the central portion of the lung, along major airways. SCC is one of the most diagnosed cancer but the survival rate is significantly better than adenocarcinoma. Squamous cell carcinomas are more common in men than in women and often, at an earlier stage than other tumors, squamous cell carcinomas cause symptoms such as coughing up blood.

Squamous cells are flat cells that line the lung airways, where cigarette smoke causes a metaplastic process, in which the epithelium passes from stratified columnar epithelium to a keratinizing paved epithelium¹⁹.

Keratinization, intercellular bridges, and a solid nested growth pattern are all characteristics of SCC. Typically, tumor cells feature defined intercellular bridges, moderate to abundant cytoplasm, visible to inconspicuous nucleoli, and hyperchromatic nuclei. Individual tumor cells may become keratinized, as well as clusters of keratinizing squamous cells that create pearl-shaped keratin structures in the center of solid tumor nests¹³.

A poorly differentiated malignant tumor, without squamous cell characteristics that resembles small-cell lung cancer, can be recognized through immunomarkers of squamous cell differentiation such as p40, p63, and cytokeratins 5/6, instead TTF-1 is negative²⁰.

Keratinizing, nonkeratinizing, and basaloid are subtypes of SCC¹⁶. Except for basaloid SCCs, which purportedly have unique genetic profiles conferring intrinsic resistance to cytotoxic treatment, such subclassification does not appear to have any predictive significance, like the adenocarcinoma one. But distinguishing squamous type from adenocarcinoma has important implications in chemotherapeutic agent choices to avoid certain complications. For instance, the use of the vascular endothelial growth factor inhibitor bevacizumab should be avoided, since it may cause a possibly fatal pulmonary hemorrhage¹³.

1.2.2.3 Large cell carcinomas

Large cell carcinoma (LCC) is a type of NSCLC diagnosed by exclusion because it is poorly differentiated and cannot be further classified by immunohistochemistry (IHC) or electron microscopy: it lacks morphologic and immunohistochemical evidence of adenocarcinoma, SCC, or neuroendocrine carcinoma. LCC represents less than 3% of lung cancers²¹.

Large cell carcinoma can develop in any area of the lung, although it typically develops in the periphery, it looks bulky and necrotic, and it has large, polygonal tumor cells with pleomorphic and vesicular nuclei that form solid sheets or nests without any discernible patterns¹³.

The diagnosis of LCC requires extensive sampling of a surgical resected specimen after ruling out SqCC, ADC, or SCLC, and therefore, it cannot be made on core needle biopsies or cytology samples. For this reason, in small biopsies or cytology material, tumors with NSCLC features and null IHC phenotype are named NSCLC-NOS (not otherwise specified)¹².

LCC could be immunohistochemically positive for cytokeratins yet negative for TTF-1 and p40. Large-cell neuroendocrine carcinoma (LCC) should be recognized from solid pattern of ADC (TTF-1 positive), non-keratinizing SqCC (p40 positive), and occasionally adenosquamous carcinoma (showing both ADC and SqCC differentiation)²².

1.2.2.4 Other

Squamous, glandular, or neuroendocrine differentiation will be present in 90% of instances, other subsets of lung cancer, with both diverse classifications and broad terminology, are also included in NSCLC. These include non-small cell neuroendocrine tumors, sarcomatoid carcinoma, and adenosquamous carcinoma, most of which have a sluggish growth rate¹³.

There are a few uncommon forms of lung cancer, including adenoid cystic carcinomas, lymphomas, sarcomas, and benign lung tumors such as hamartomas. But this topic is not going to be further explained since it is handled differently from the most typical kinds of lung cancer.

1.3 Diagnostic process

When a diagnosis of lung cancer is suspected, it is required a complete medical history, and it also must be noted weight loss, performance status (PS), comorbidities, smoking history, and physical examination.

During the diagnosis stage, imaging methods and biopsy screening strategies are essential for the staging of the tumor. Additionally, having laboratory tests performed is typically indicated, particularly those that analyze the patient's blood, kidney, and liver functions.

Early detection is necessary in order to administer treatment, which may avoid cancer-related death. The effectiveness of screening is based on how quickly cancer can be identified and how many deaths can be prevented by early intervention, as opposed to later symptom-driven diagnosis and intervention.

Screening for cancer is a repetitive process, starting with the initial diagnostic test, followed by repeat rounds of investigations.

1.3.1 ECOG Performance status (PS)

The physical examination is a fundamental step to determine the Performance status: a prognostic tool, which aids in the choice of treatment (surgery, radiation, chemotherapy) or the intensity of palliative treatment in cancer patients²³.

PS is based on an assessment of the patient's general conditions, and it is a condensed version of the Karnofsky performance score (KPS). The Karnofsky index, between 100 and 0, has been simplified into a five-point scale where 0 represents a fully active patient instead 5 implies that the patient has died as reported in the Table 1.3.1.

Although PS is short, easily understood and part of the global language of oncology, it is entirely subjective and depends on the experience and opinion of the oncologists²⁴. Moreover this score fails to account for multimorbidity, frailty or cognition, even now, poor PS is one of

the requirements for being eligible for most cancer treatments and records patient fitness for treatment.

Grade	ECOG performance status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

Table 1.3.1 WHO/ ECOG performance status scale.

1.3.2 Staging

The Tumor-Node-Metastasis (TNM) system has become a widely used method of describing the anatomic spread of cancer. TMN staging system considers the size, location, and extent of the primary tumor (T descriptor), the presence and location of lymph node involvement (N descriptor), and the presence or absence of distant metastatic disease (M descriptor) as shown in the Table 1.3.2.

This common language performs many functions, including providing some prognostic indications, assisting clinicians in treatment planning, aiding in the evaluation and comparison of treatment results, and facilitating information sharing between various treatment centers²⁵.

Most often, computed tomography (CT) imaging, used to estimate tumor size, determines the T descriptor with $T1a \leq 2$ cm, $T1b > 2$ but ≤ 3 cm, $T2a > 3$ but $5 \leq$ cm, $T2b > 5$ but ≤ 7 cm, and $T3 > 7$ cm²⁶.

The most significant factor affecting care and prognosis is nodal status, which comes second only to the exclusion of distant metastases. Patients with verified mediastinal (N2) nodal involvement are typically considered for a different therapeutic approach, whereas patients with node-negative clinical stage I or stage II are given a drastic curative treatment.²⁷

Nodal involvement is described using the N descriptor, with N0 representing no nodal involvement. N1 denotes the presence of metastasis into 10–14 lymphoid stations that is the

ipsilateral peribronchial or perihilar lymph nodes and intrapulmonary nodes. N2, which represents lymph node stations from 2 to 9, describes tumor metastases or direct extension into ipsilateral mediastinal or subcarinal lymph nodes. N3 status denotes metastases into station 1 supraclavicular nodes or contralateral mediastinal, contralateral hilar, ipsilateral, or contralateral scalene nodes²⁶.

M classification is broken into three categories: category M1a (intrathoracic disease), M1b illness (single extrathoracic metastasis), and M1c disease (multiple thoracic metastases)²⁷.

An inaccurate staging can influence a judgment regarding appropriate treatment recommendations. Curative surgical resection is beneficial for patients with stage IA, IB, IIA, and IIB NSCLC, but it is rarely beneficial for individuals with stage IIIA, IIIB, and IV cancer²⁸. Clinical assessment and computed tomography (CT) can give a first estimate of the severity of the disease, but in most situations, the stage needs to be validated with additional testing.

TNM staging system	
Primary tumor (T)	
TX	Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualised by imaging or bronchoscopy
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e. not in the main bronchus) ^a
T1a	Tumor 2 cm or less in greatest dimension
T1b	Tumor >2 cm but 3 cm or less in greatest dimension
T2	Tumor >3 cm but 7 cm or less or tumor with any of the following features (T2 tumors with these features are classified T2a if 5 cm or less); involves main bronchus, 2 cm or more distal to the carina; invades visceral pleura (PL1 or PL2); associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T2a	Tumor >3 cm but 5 cm or less in greatest dimension
T2b	Tumor >5 cm but 7 cm or less in greatest dimension

T3	Tumor >7 cm or one that directly invades any of the following: parietal pleural (PL3) chest wall (including superior sulcus tumors), diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium or tumor in the main bronchus (<2 cm distal to the carinaa but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung or separate tumor nodule(s) in the same lobe)
T4	Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, oesophagus, vertebral body, carina, separate tumor nodule(s) in a different ipsilateral lobe
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Separate tumor nodule(s) in a contralateral tumor with pleural nodules or malignant pleural (or pericardial) effusionb
M1b	Distant metastasis

Table 1.3.2 Classification of descriptors used for TMN staging.

Anatomic stage/prognostic groups according to the AJCC/UICC TNM staging system			
	TX	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1a,b	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T2b	N0	M0
	T1a,b	N1	M0
	T2a	N1	M0
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1a,b; T2a,b	N2	M0
	T3	N1, N2	M0
	T4	N0, N1	M0
Stage IIIB	T4	N2	M0
	Any T	N3	M0
Stage IV	Any T	Any N	M1

Table 1.3.2 Classification of anatomic stage according to the AJCC/UICC TNM staging system.

1.3.3 Imaging techniques

Lung cancer clinical staging involves radiology and nuclear medicine to assess the fundamental variables of suspecting neoplasia (size, shape and growth over time). After a proper medical history and physical examination, an x-ray of the chest usually is the first step to confirm a suspicion of neoplasm. However, according to the AIOM lung cancer guidelines, a CT (computed tomography) scan of the chest, abdomen, and lower cervical region should be the next step in the further examination²⁹. Positron Emission Tomography (PET) imaging, particularly the integrated 18-FDG-PET-TC approach, is used to complete staging when the CT scan does not reveal the existence of distant metastases²⁸. PET is a nuclear medical research approach that uses radiopharmaceuticals that release positrons to give a precise clinical picture of the degree and metabolic activity of the disease. Fluorodeoxyglucose (18F-FDG) allows for a precise evaluation of a tumor's metabolic behavior, which is frequently connected to the tumor's level of aggressiveness or differentiation.

Since PET-CT with 18F-FDG is more sensitive to detecting extrathoracic and bone metastases than CT, it enables more precise staging of lung cancer²⁷, thanks to the evaluation of cellular metabolism, which usually precedes anatomical changes.

Before excluding potentially operable patients from surgery, it is necessary to investigate the relative incidence of false positives on CT scans using ultrasonography and MRI (Magnetic Resonance Imaging) and if there is still a question, a histological check may also be necessary. As well as a confirmation tool, MRI is the elective diagnostic examination for the evaluation of metastatic involvement of the central nervous system and it offers better results than CT in the evaluation of parietal pleural and chest wall invasion³⁰.

1.3.4 Sampling procedures

The location of the initial tumor (central or peripheral), the growth pattern of the airway (endobronchial versus peribronchial), and the presence of mediastinal and/or distant lymph node metastases all determine the most appropriate invasive method for histologically categorizing the disease.

Most of the time, fiberoptic bronchoscopy is used to type central tumors, which are endoscopically visible or located in the inner of the lung³¹. So whether the lesion is defined as endobronchial or endotracheal, and the bronchial mucosa is intact, it is biopsied with normal flexible forceps or, if the surface is severely necrotic, with transbronchial needle aspiration (TBNA). TBNA, bypassing the surface and samples the lesions more deeply, may reduce the risk of failing to obtain viable tissue when the surface of the lesion appears white due to possible significant necrosis.

In the endobronchial pattern and peribronchial pattern with airway compression, a blind transbronchial needle aspiration procedure can also be carried out. Instead in the peribronchial pattern with no airway compression, the lesion is totally invisible at bronchoscopy. In this situation, the best way to maximize diagnostic yield and minimize potential problems is to use ultrasound guidance.

On the other hand, in cases where the central tumor is located close to a medium- to large-caliber airway or near the esophagus but does not expand within the airway (peribronchial/esophageal pattern), it can be biopsied in real-time by transbronchial ultrasonography (EBUS, endobronchial ultrasound), or transesophageal ultrasound (EUS, endoscopic ultrasound).³²

Despite the current availability of extremely advanced tools for guiding bronchoscopic samples, the diagnostic management of peripheral lesions, which are located in the outer third of the lung and not endoscopically visible, is certainly more complicated and variable.

As a result of technological advancement, several practical guidance systems, including electromagnetic navigation, virtual bronchoscopy navigation, and radial EBUS, have been introduced to the market. These systems may be used to successfully direct the bronchoscopy sampling tools (forceps, needles, and brushes) towards peripheral pulmonary nodules for precise biopsy³¹. The disadvantages of these new techniques are that they need a lot of time and money, as well as the requirement for the use of mild to deep sedation.

Evidence from the literature suggests, in very peripheral lesions, in lesions < 2 cm, and in lesions that are predominantly or exclusively ground-glass, the percutaneous transthoracic CT-guided approach (TTNA), is certainly much more effective than the endoscopic approach, although it carries a higher risk of pneumothorax.

Regarding the staging of the N parameter, transbronchial needle biopsy (TBNA) is a method used to sample the low paratracheal (4R, 4L), subcarinal (7), and hilar (10R, 10L, 11R, 11L) lymph node stations³³.

Bronchial ultrasound (Endo-Bronchial Ultrasound, EBUS) allows for observing the penetration of the needle in real-time but is unable to sample lymph nodes that are not close to the airway (e.g. stations 8 and 9). The mediastinal nodes next to the esophagus, including those in the inferior mediastinum, as well as the liver and left adrenal metastases, were sampled using EUS-fine needle aspiration (FNA)²⁸.

A single biopsy can also provide staging data, such as the exclusion of central and mediastinal lymph node lesions, which is crucial for patients who may be candidates for surgery.

1.3.5 Neoplasm typing

Squamous cell carcinoma (CS), adenocarcinoma (ADC), large cell carcinoma (LCC), and small cell carcinoma represent the majority of lung cancers, accounting for about 95% of cases. The precise histological definition of lung carcinomas is conducted by evaluating conventional morphological criteria using routine hematoxylin and eosin (H&E). But if the cytomorphologic features are not clear, a limited IHC panel of generally mutually exclusive markers is recommended³⁴.

In poorly differentiated NSCLC without neuroendocrine morphology, to preserve the neoplastic tissue for predictive molecular investigations, the minimum basic panel suggested by the WHO

is TTF-1 (Thyroid Transcription Factor-1, marker of ADC) and p40 (marker of CS); If neuroendocrine features are present, the markers to be used to confirm neuroendocrine differentiation are chromogranin, synaptophysin and CD56³⁵.

Usually, immunohistochemical testing (IHC) should be conducted using tissue blocks obtained by biopsy or surgical resection but in clinical practice, most patients frequently have access to small sample sizes (such cytology samples). For this reason, the histotype definition can be defined both on biopsy samples fixed in formalin, and on cytological preparations.

Anyway, before performing immunohistochemical tests, biopsy samples are placed in 10% neutral buffered formalin to reduce the impact of cold ischemia on the tissue to avoid DNA damage in pre-analysis.

About the cytological material obtained during simple/guided needle aspiration procedures (transbronchial, transthoracic, transesophageal) or in effusions (pleural or extra-pleural), the preparation of cytoinclusions in paraffin blocks (cell-blocks) is very useful, especially in view of the possibility of analyzing by IHC some of the biomarkers predictive of response to medical therapy, such as Programmed Death-Ligand 1 (PD-L1).

It is simpler and more reliable to conduct molecular investigations (immunohistochemistry) for diagnostic (e.g., TTF-1 and p40) and predictive (e.g., ALK, ROS1, PD-L1) purposes using the cytoinclusion preparation in addition to conventional cytological smear preparations.

Even if the cytological sample is set up as a smear or thin layer, it offers higher quality than the cell-block, especially in terms of fixation artefacts and length of nucleic acid fragments.

1.3.6 Molecular analysis

A revolutionary shift in therapy targeting and monitoring has been made possible by the molecular study of lung cancer gene mutations. The assessment of specific genetic alterations has proven to be crucial in predicting treatment response and it is a key element in the choice of molecularly targeted treatments.

In this regard, the AIOM guidelines recommend performing the morphological diagnosis and complementing it with the characterization of mutations in EGFR (Epidermal Growth Factor Receptor) and BRAF (B-Raf proto-oncogene), the definition of translocations in ALK (Anaplastic Lymphoma Kinase), ROS-1 (Proto-oncogene tyrosine-protein kinase) and NTRK 1,2 and 3 (Neurotrophic Tyrosine Receptor Kinase) and the assessment of PD-L1 (Programmed-death ligand 1)²⁹.

EGFR, ALK, ROS1, and programmed death ligand 1 are among the predictive molecular biomarkers that are first assessed for lung cancer because they are currently approved and reimbursed by the Italian healthcare system³⁶.

Given the limited material available for predictive molecular pathology testing and the ability to simultaneously analyze EGFR and BRAF alterations as well as ALK, ROS1, and NTRK translocations, next-generation gene sequencing (NGS) is recommended over conventional technologies for the evaluation of molecular biomarkers. However, it is noted that due to the complexity of the technology and the high costs, NGS must be implemented in centers prepared to handle the sample. Furthermore, a considerable number of molecular pathology labs have a next-generation sequencing platform, but just a few big-volume centers have it implemented in a clinical context. The main problems associated with this heterogeneous scenario can be summarized, by considering the differences between reference ranges (numbers and types of mutations detected) and limits of detection (the lowest quantity of mutated alleles detected), which lead to a different mutation rate and specific variant distribution.

Another significant issue concerning the implementation of pertinent biomarkers analysis for patients with NSCLC in the clinical context is related to the differences in the regional reimbursement systems. Indeed, some Italian regions still lack specific reimbursement codes and request procedures for EGFR, ALK, ROS1, and PD-L1 testing. For the centralized laboratories receiving tumor samples from different institutions, the lack of standardization in both test requests and reimbursement procedures represents an urgent problem from the administrative point of view.

Since immune checkpoint inhibitors are the main topic of this thesis, two main biomarkers, that must be screened to define appropriate prescription of these antibodies, driver mutations concerning EGFR and ALK rearrangements will now be reviewed.

1.3.6.1 Evaluation of EGFR mutation

The EGFR gene is situated at location 12 on the short arm of chromosome 7. The transmembrane glycoprotein that this gene encodes belongs to the protein kinase superfamily. These mutations increase the kinase activity of EGFR, which leads to hyperactivation of prosurvival signaling pathways. First generation (gefitinib and erlotinib), second generation (afatinib, dacomitinib), and third generation (osimertinib) EGFR tyrosine kinase inhibitors (EGFR TKIs) are highly effective in treating tumors with EGFR mutations, but the majority of patients relapse and develop resistance, which is most frequently linked to a second mutation in exon 20³⁷.

In 40% and 80% of NSCLC cases and numerous other epithelial malignancies, EGFR is overexpressed. Lung cancers connected to EGFR mutations are seen in 35% of NSCLC patients in East Asia and 10% of NSCLC patients in the United States. Approximately 90% of these mutations result in exon 19, deletions CTG to CGG, or exon 21 at nucleotide 2573, that results in substitution of leucine by arginine at codon 858 (L858R)³⁸.

Patients with NSCLC with ADC, LCC, NSCLC mixed with ADC and NSCLC N.A.S. histotypes, which have the highest probability of mutation detection, can be submitted to EGFR mutational analysis; instead in cases of "pure" squamous carcinoma (p40 +/TTF1-), the patient may not be tested as EGFR is almost certainly not mutated, except the rare cases of squamous carcinoma in young or non-smoking patients, where the test should be performed anyway. Furthermore, in cases of squamous cell carcinoma diagnosed on small tissue biopsies or cytological specimens, testing is still recommended, as the presence of a mixed (adeno/squamous) component cannot be excluded³⁹.

Determination of EGFR mutations can be performed on the surgical specimen or on biopsy or cytological sampling of the primary tumor and/or metastasis. In Italy, various practices are now in use: the most popular method is real-time polymerase chain reaction, which is followed by Sanger sequencing, pyrosequencing, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and pyrosequencing⁴⁰.

In non-smokers, light smokers (<15 packets/year or ≤5 cigarettes/day) and ex-smokers (since ≥15 years), in whom standard lung biopsy cannot provide sufficient tissue/cytological material for molecular analysis, analysis of EGFR exons 18, 19, 20 and 21 alterations on circulating tumor DNA (ctDNA) extracted from peripheral blood (plasma) is indicated.

Liquid biopsy is currently recommended as a possible alternative to tumor tissue analysis in patients in whom the quantity and/or quality of available tissue is insufficient to perform the intended molecular analysis⁴¹.

1.3.6.2 Evaluation of ALK rearrangements

ALK oncogene rearrangements with EML-4 or other fusion partners on the short arm of chromosome 2, at position 23, results in the production of a particular protein with tyrosine kinase activity that is involved in the processes of cell survival and proliferation and that consists of the COOH-terminal kinase domain of ALK and the NH2-terminal portions of different genes.⁴² About 3-7% of pulmonary lung tumors have chromosomal rearrangements involving the ALK gene's tyrosine kinase domain, which identifies a subgroup of patients who

are candidates for treatment with first-generation (crizotinib), second-generation (alectinib, ceritinib, brigatinib), and new generation (lorlatinib) ALK tyrosine kinase inhibitors⁴⁰.

The diagnostic reference investigation for the determination of ALK was FISH (Fluorescence In Situ Hybridization) and reverse transcription polymerase chain reaction (RT-PCR), although in recent years, the evidence supporting the use of IHC has increased considerably. For the FISH test, a cut-off of 15% of rearranged neoplastic cells is required to express positivity⁴³.

1.3.6.3 Evaluation of PD-L1

Evaluation of PD-L1 expression is indicated in patients with NSCLC with ADC histotype, CS, LCC, mixed NSCLC with ADC and NSCLC N.A.S. Analysis of PD-L1 expression can be performed on the surgical specimen, or on biopsy or cytology specimen of the primary tumor and/or metastasis; the cytology specimen must be fixed in formalin and embedded in paraffin (cell-block)⁴⁴.

Evaluation of PD-L1 expression should be performed by IHC with validated antibodies for formalin-fixed and paraffin-embedded samples. Before analyzing samples in IHC, it is mandatory to assess the adequacy of the preparation. To date, the only quantitative parameter derived from the inclusion criteria of clinical studies that have evaluated this specific biomarker is the number of neoplastic cells present, which must be no less than 100⁴⁴.

About the need for which the PD-L1 test is performed in clinical practice, the only clinically validated way of interpreting the result involves the application of the tumor proportion score (TPS). This is based on the percentage assessment of PD-L1 positivity at the neoplastic cell membrane, even when this is partial. It does not take cytoplasmic and immune cell positivity into account⁴⁴.

The PD-L1 test report must contain the following information: type of sample analysis; protocol and platform used (with reference to the validation procedure if no CE-IVD diagnostic devices are used); microscopic evaluation of the sample to define adequacy; Tumor Proportion Score (TPS) for PD-L1, as defined above. Given the criteria on which the TPS is based, it is not necessary to report staining intensity information (e.g. 1+, 2+, 3+), because a cell with partial membrane staining and low intensity for PD-L1 should also be considered positive. Where possible, it is also preferable to report a point estimate of the PD-L1 expression rate defined according to TPS.

1.4 Management of lung cancer therapies

The treatment of lung cancer is decided according to clinical stage, morphological diagnosis, and the performance status of the patient and it has changed markedly in recent years: surgery, chemotherapy, targeted therapy, and radiotherapy are just a few of the treatment options that have significantly improved this disease's prognosis.

For decades, the standard of care treatment for advanced stage NSCLC included only cytotoxic chemotherapy but in these years the landscape of treatment has rapidly evolved as a result of two major treatment milestones: targeted therapy and immunotherapy.

Targeted therapy using drugs specifically designed to inhibit mutation-driven genetic alterations affords more effectiveness and less toxicity than generic chemotherapeutic agents and therefore substantial improvement of outcomes compared with standard chemotherapy in the treatment of advanced NSCLC. One of the common mechanisms of carcinogenesis is constitutive activation of receptor tyrosine kinases, such that inhibition of their activity creates an effective modality for anticancer therapy. With the advent of tyrosine kinase inhibitor (TKI) treatments, it is important to screen patients with lung cancer for actionable gene mutations. EGFR mutation and ALK translocation are the most effectively targeted oncogenes in NSCLC and are now considered standard of care⁴⁵. Recent advancement in testing methodologies such as next-generation sequencing (NGS) affords multiplex systems to detect multiple gene alterations on one single platform. Non-invasive plasma and serum-based DNA detection and monitoring are emerging molecular tools.

Immunotherapy has been changing the way NSCLC is treated in many settings since 2015 and has helped these patients improve their lives. Immune checkpoint inhibition-based immunotherapy, which focuses on PD-1 and CTLA-4, is now the most popular immunotherapy approach in clinical practice.

1.4.1 Elderly patients

According to ESMO clinical practice guideline, early-stage patients are typically indicated for surgery with the addition of post-operative chemotherapy (ChT). Adjuvant ChT should be provided to individuals with stage II and stage III NSCLC and it can be taken into consideration in those with stage IB disease and a primary tumor larger than 4 cm⁴⁶. For adjuvant ChT, a two-drug combination with cisplatin, delivered in three to four cycles, is preferable. But for those patients not eligible for doublet chemotherapy, single-agent chemotherapy remains the standard of care.

In the current state of knowledge, targeted agents should not be used in the adjuvant setting while (Neo)adjuvant anti-PD(L)-1 checkpoint inhibitors are currently being evaluated in addition to current standard of care⁴⁷.

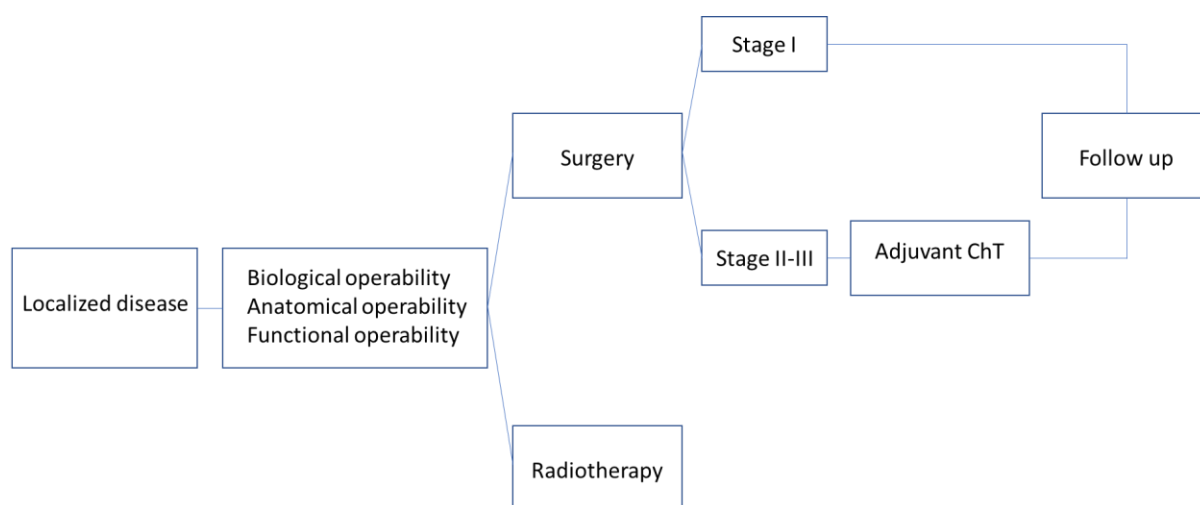


Figure 1.4.1 Schematic representation of the management of disease in the early stages.

Stage IIA, IIB, and patients who cannot or will not undergo surgery are candidates for exclusive radiotherapy treatment if cN0; otherwise, chemo-radiotherapy is the recommended when cN1²⁹.

1.4.2 First-line treatment of EGFR and ALK-negative disease

Whether the optimal treatment for all patients with locally advanced, but surgically resectable, NSCLC remains chemoradiation, for patients with unresectable or inoperable locally advanced disease, the incorporation of immunotherapy consolidation after chemoradiation has defined a new standard of care. Indeed all stage IV NSCLC patients with EGFR- and ALK-negative disease, without significant co-morbidities, and PS 0-2 should be given chemotherapy an immunotherapy consideration.

In patients with PD-L1 > 50% treatment consists of pembrolizumab monotherapy. In the category of patients with PD-L1 < 50% to non-squamous histology, four cycles of pembrolizumab plus platinum-based chemotherapy plus pemetrexed are the recommended option. In patients with PD-L1 < 50% with squamous cell histology, four cycles of pembrolizumab are recommended, along with chemotherapy based on carboplatin plus paclitaxel²⁹.

For non-squamous cancers and patients on third-generation regimens, cisplatin should be the first choice. While the nab-paclitaxel (nab-PC) regimen could be an alternative for patients with advanced NSCLC who are at higher risk of neurotoxicity, have a history of paclitaxel

hypersensitivity, or have other conditions that exclude the use of normal paclitaxel premedication. Pemetrexed is only used for NSCLC in any line of therapy, and in patients with non-squamous tumors, it is preferred over gemcitabine or docetaxel⁴⁷.

It is advised to use platinum-based doublets in advanced SCLC patients together with a third-generation cytotoxic drug (gemcitabine, vinorelbine, taxanes), followed by 4 cycles of durvalumab or atezolizumab. Etoposide and cisplatin or etoposide and carboplatin are still regarded as conventional chemotherapy combinations⁴⁷.

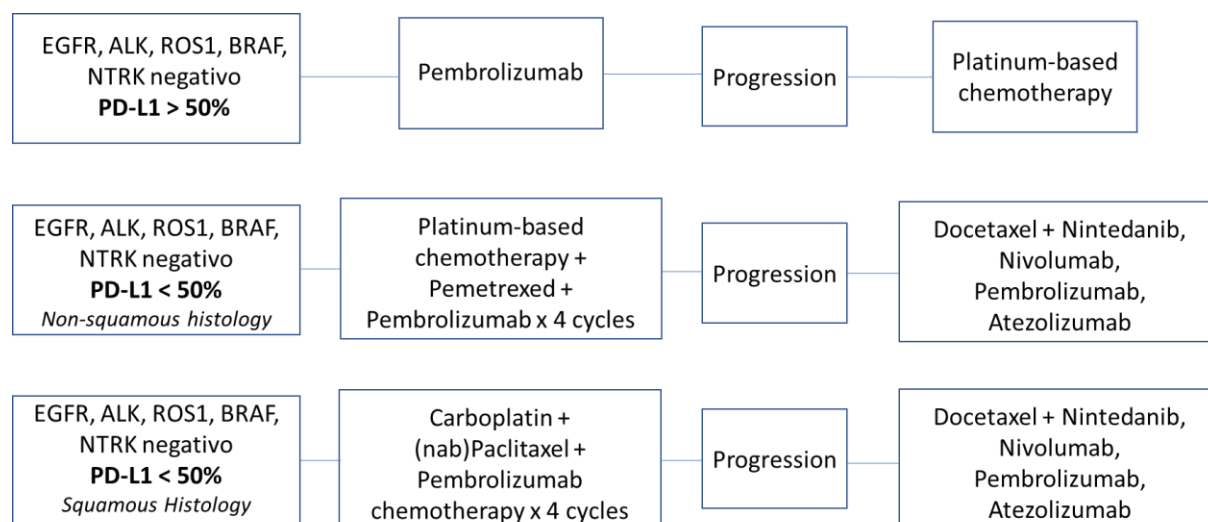


Figure 1.4.2 Schematic representation of the management of the metastatic disease NON oncogene addicted.

1.4.3 PS 2 and beyond

In patients with PS 2, carboplatin-based combination chemotherapy compared with best supportive care (BSC) prolongs survival and improves the quality of life, unlike poor PS (3–4) patients who should be treated with BSC only⁴⁷. Single-agent chemotherapy with gemcitabine, vinorelbine and docetaxel is an alternative treatment option to carboplatin therapy.

1.4.4 Second-line treatment of EGFR- and ALK-negative disease

Patients who have progressed clinically or radiologically following first-line treatment with PS 0-2 should be provided second-line chemotherapy that includes docetaxel or pemetrexed (for NSCLC alone).

Patients clinically or radiologically progressing after first-line of immunotherapy receive different treatments depending on PD-L1 expression: PD-L1 >50%, second-line therapy involves platinum-based chemotherapy. Patients who have PD-L1 <50%, the treatment choices are a combination of Docetaxel plus Nintedanib, whereas if they have not received

immunotherapy in the first line, the guidelines recommend treatment with Nivolumab, Pembrolizumab, Atezolizumab²⁹.

Nivolumab at 3 mg/kg every 2 weeks is recommended also in pretreated patients with advanced SCLC, and as mentioned before, it represents, such as Atezolizumab, a treatment option in pretreated PD-L1-positive patients with advanced NSCLC. Nivolumab and Atezolizumab compared to docetaxel, showed better results and a lower toxicity profile⁴⁸.

Nintedanib combined with docetaxel is a treatment option in patients with adenocarcinoma, especially in those progressing within 9 months from the start of first-line chemotherapy or immunotherapy.

Pembrolizumab at 2mg/kg every 3 weeks is recommended in pretreated patients with platinum-pretreated, advanced SCLC or NSCLC expressing PD-L1.

Erlotinib may be an option for patients who have WT EGFR or unknown EGFR status and are not candidates for chemotherapy.

Afatinib may be an alternative for SCLC patients with PS 0-2 who are ineligible for chemotherapy and have an unclear EGFR status or an EGFR WT mutation.

1.4.5 Maintenance

Only patients with a PS of 0-1 should get maintenance therapy after receiving first-line chemotherapy. The histology, responsiveness to platinum-doublet chemotherapy, residual side effects from first-line therapy, PS, and patient preference should all be considered in determining whether to continue with maintenance. In patients with NSCLC and PS 0-1 who have disease control after four rounds of cisplatin-pemetrexed should be given consideration pemetrexed continued maintenance⁴⁷.

Erlotinib is indicated for switch maintenance treatment but limited to patients with locally advanced or metastatic NSCLC with EGFR-activating mutations.

1.4.6 Tumors with an activating EGFR mutation

First-line treatment with an EGFR TKI (osimertinib, erlotinib, gefitinib or afatinib) is the standard of care for tumors bearing an activating (sensitizing) EGFR mutation. Patients with EGFR mutation and PS 3-4 may also be offered an EGFR TKI. Since head-to-head analyses of these medications show that osimertinib is the most effective medication, it represents the first choice of treatment⁴⁶.

If the information on an EGFR-sensitizing mutation becomes available during first-line platinum-based chemotherapy, continuing chemotherapy for up to four cycles and offering the

EGFR TKI as maintenance treatment in patients achieving disease control, or as second-line treatment at the time of progression, is the better treatment choice⁴⁶.

In patients who progress after an EGFR TKI, rebiopsy is strongly encouraged to look for EGFR T790M mutation, relevant for therapeutic strategy. An alternative to tissue rebiopsy is represented by liquid biopsy. In patients who have developed the EGFR T790M resistance mutation after EGFR TKI treatment, osimertinib is recommended. When a rebiopsy is not feasible, or when the EGFR T790M mutation is not detected in patients who progress after an EGFR TKI, the standard of care is platinum-based doublet chemotherapy. No data support the concurrent use of EGFR TKI and platinum-based doublet chemotherapy⁴⁷.

1.4.7 Tumors with ALK rearrangement

First-line treatment with alectinib or brigatinib is preferred for patients with ALK-rearranged NSCLC, also taking ceritinib and crizotinib into consideration for the first line⁴⁷.

In patients who progress after an ALK TKI, second-generation ALK inhibitors such lorlatinib, alectinib, ceritinib, brigatinib are recommended.²⁹ Head-to-head trials comparing crizotinib with alectinib, brigatinib, or lorlatinib have been conducted to determine the best ALK inhibitor. Each of these studies found that the second- or third-generation agents (alectinib, brigatinib or lorlatinib) were more effective than crizotinib⁴⁶.

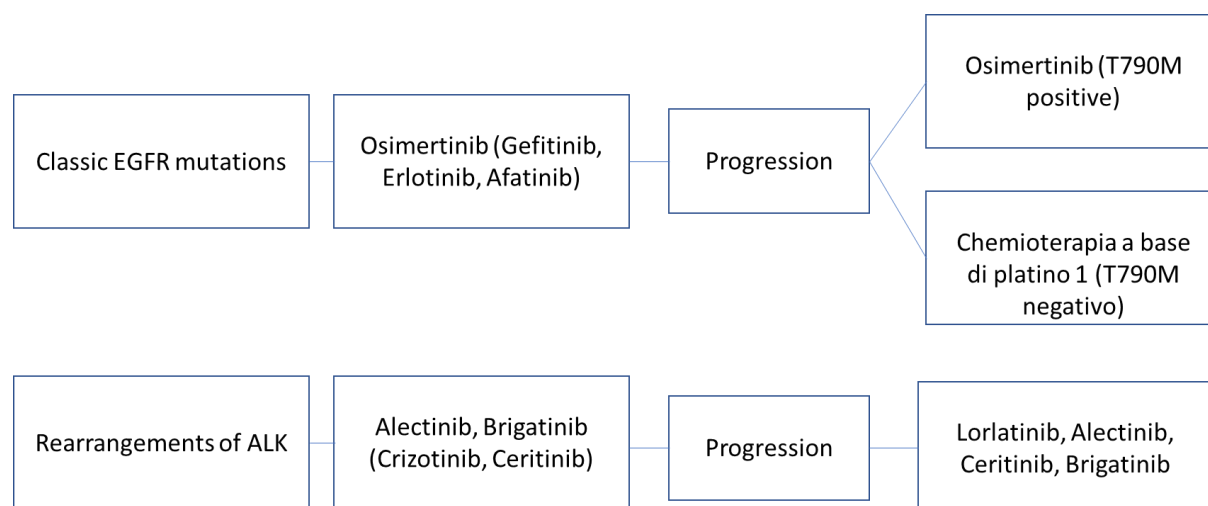


Figure 1.4.7 Schematic representation of the management of the metastatic disease oncogene addicted.

1.4.8 Role of radiotherapy

Radiotherapy can achieve symptom control for bone and brain metastases and is also effective in treating pain related to chest wall, soft tissue or neural invasion.

Radiotherapy is an integral part of treatment of either type of lung cancer and it is indicated in cases of haemoptysis, symptomatic airway obstruction and following surgery for CNS, and, sometimes, bone surgery⁴⁷.

1.4.9 Response evaluation

Response evaluation is recommended after two to three cycles of therapy using the same radiographic investigation that initially demonstrated tumor lesions.

Measurements and response assessment should follow RECIST criteria. However, the adequacy of RECIST in evaluating the response to EGFR or ALK TKI in respective genetically driven NSCLC is debatable. In the case of immune checkpoint inhibitor therapy, RECIST criteria should be used, although irRC may have a role in the overall assessment of therapy⁴⁹.

1.5. Immune Checkpoint Inhibitors (ICI)

Immunotherapy, either as monotherapy or in combination, has become the gold standard of care in the first-line setting for advanced squamous and non-squamous lung cancer in patients without targetable genetic changes and without contraindications to PD-1/PD-L1 inhibitors. The anti-PD-1 pembrolizumab and nivolumab, anti-PD-L1 inhibitor atezolizumab, and the anti-CTLA4 inhibitor ipilimumab are the checkpoint inhibitors used in advanced NSCLC.

All of them are monoclonal antibodies (mAb) which provide considerable advantages over conventional cytotoxic chemotherapy in terms of reduced toxicity, decreased size of solid tumors, inhibition of advanced cancers and metastases, and improved patient survival⁵⁰.

Checkpoint inhibitors are linked to a particular variety of toxicity known as immune-related adverse effects (irAEs). This results from the activation and subsequent infiltration of immune cells into normal tissue and to its long half-life and binding time with the target⁵¹. These immune mediated inflammation can affect any part of the body, such as gastrointestinal tract, lung, liver, and pituitary gland inducing colitis, pneumonitis, and endocrine dysfunction⁴⁶.

1.5.1 Mechanism of action of Immune Checkpoint Inhibitors (ICIs)

In the immune systems T cells have receptors that scans cells to distinguish between normal cells and abnormal. Once detected, abnormal cells are attacked and remove from T cells but sometimes T cells, thanks to evasion mechanisms of cancer, doesn't identify these mutated cells. Cancer cells get past the security system with the help of PDL-1, that is found in the surface of the cancer cells. PDL-1 in normal conditions help maintaining immune homeostasis, but when is abundantly expressed in the neoplastic mass, because it can be upregulated by

interferon gamma (IFN- γ) produced by activated T cells, it allows the tumor to remain undetected⁵².

PD1 is a checkpoint protein and it belongs to the CD28 family which regulates various aspects of immune functions. PD1 is a type I transmembrane protein expressed in immune cells such as T, B, NK cells, monocytes, and dendritic cells (DCs). It pertains to a class of suppressor T-cell receptors that were upregulated by antigen stimulation and cytokines brought on by T cell activation⁵³.

PDL1 is a transmembrane glycoprotein that is expressed by a variety of cell types, including cancer cells, and belongs to the B7 family of co-stimulatory/coinhibitory molecules of antigen presentation. It is advantageously situated to control T cell activity in DCs and other antigen-presenting cells (APCs). The cytokines produced by the encounter between the T cell and the antigen, in addition to amplifying the inflammatory process, stimulate the expression of PD-L1 on the tissues. This explains why cancer cells upregulate PD-L1 when they recognize the PD1 protein on T lymphocytes, increasing the chances of binding between PD1 and PD-L1 and causing the T cells to undergo apoptosis⁵⁴.

Additionally, it inhibits tumor-infiltrating CD4+/CD8+ T cells (CD4+/CD8+ TILs) limiting cytokine production, such as tumor necrosis factor (TNF), interferon-gamma (IFN-), and interleukin-2 (IL-2), allowing cancer cells to avoid the immune response⁵⁵.

In order to achieve tumor immune escape, the PD1/PDL1 signal transduction pathway is a crucial part of tumor immunosuppression. It can prevent T lymphocyte activation and enhance tumor cell immune tolerance. In conclusion, PD1 binding to PDL1 can reduce T cell-mediated immune surveillance, causing a lack of immunoreaction and potentially T cell apoptosis.

PD1/PDL1 inhibitors unblock the immune suppression of anti-tumor T cells (Figure 1.5.1), which results in T cell multiplication and permeation into the tumor microenvironment and inducing an anti-tumor response⁵⁶. AntiPD1/PDL1 treatment now available prevents the

interaction of PD1 and PDL1, as well as activating depleted immune cells and inducing an immune response that avoids the cancer escape.

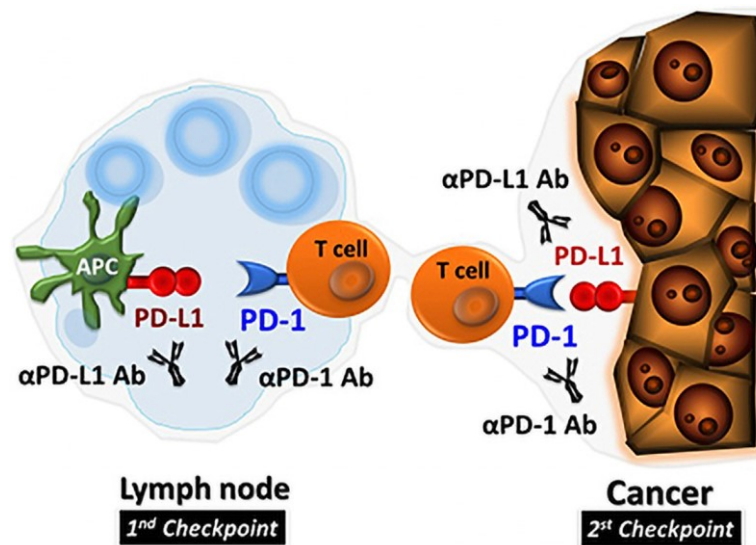


Figure 1.5.1 PD1/PDL1 inhibitors in TME (Hamanishi et al., 2016).

1.5.2 Response to ICI treatment

Contrary to conventional cancer therapy, checkpoint inhibitors may cause unusual patterns of response, for which evaluation is more difficult. prognosis In ICI treatment the delayed response, the appearance of new lesions or the growth of tumor are not necessarily associated with a negative prognosis. A few weeks may pass before mounting an effective anticancer immune response, and this delay may cause the tumor to develop initially before regressing⁵⁷. The initial stages in which the tumor mass expands can reflect tumoral lymphocyte infiltration or poor immune response: this procedure is known as pseudo-progression and there are currently no definitive radiologic criteria to distinguish it.

As a result, there are many disadvantages to using RECIST criteria for tumor response assessment in this circumstance, such as its inability to discriminate between pseudo-progression and progression⁵⁸.

Despite the difficult assessment of progression and the low response rate, immune checkpoint blocking medication has had considerable success in the clinic. But only 10 to 30 percent of patients can exhibit sustained efficacy after taking PD1/PDL1 inhibitors⁵⁹. The majority of patients have no obvious response to the treatment or will remain resistant to it⁵⁷.

The development of PD1/PDL1 antibody resistance involves many tumor-related processes, including PD-L1 expression, tumor neoantigen expression and delivery, related cellular

signaling pathways, tumor microenvironment, and epigenetic modifications. The lack of tumor antigens causes T cells to fail to recognize PD1/PDL1 antibodies, leading to drug resistance. In addition, molecules that process and deliver antigens, such as MHC class I molecules and $\beta 2$ microglobulin, can also lead to resistance to immune checkpoint inhibitors (ICIs) when their genetic code is altered⁵⁴.

After failure to respond or progression, continuation of anti-PD-1 is generally ineffective in NSCLC. Recent retrospective exploratory analysis suggests that only a few patients (8.3%) receiving treatment past progression obtain a subsequent partial response⁵¹. Unlike targeted therapy and chemotherapy, response to checkpoint inhibitor can be durable, conferring a better outcome. Therefore, it was proposed that 1- or 2-year OS may be a better indicator of efficacy than median OS.

1.5.3 Eligibility criteria

Improvements in overall survival relative to standard chemotherapy have been observed in the first-line and second line therapy settings for patients with advanced non–small cell lung cancer (NSCLC) who are treated with immune checkpoint inhibitors. But it is clear that the benefit from therapy is not universal, and identification of biomarkers to select therapy has assumed importance. In an era of increasing costs of care and potential for toxicities related to immune checkpoint inhibition, proper patient selection is critical to the optimal use of this new class of agents⁵¹.

These innovative drugs are expensive, complex to produce, and difficult to store and transport. Taking into account the costs and therapeutic relevance of these monoclonal antibodies (mABs), monitoring registers have been activated by AIFA in order to guarantee equal and homogeneous access and to ensure their appropriate use⁶⁰. To avoid clinical and financial relapses, it is essential to monitor the prescribing process and confirm that patients present specific eligibility requirements, especially in the oncological field. Registers present a tool, developed by AIFA, to control the prescriptive appropriateness and the drug costs. The most recent regulations have also given to the registers the ability to assess drug efficacy, for the purposes of renegotiation and for controlling expenditure on innovative drugs. The selection criteria for these drugs, which the clinician must assess before prescribing them, are summarized in the monitoring registers. There are multiple monitoring registers for the same drug because ICIs have multiple therapeutic indications for different tumor forms, as shown in Table 1.5.3.1.

NSCLC registries have multiple updates, only the latest versions will be reported in this study.

PATHOLOGIES FOR WHICH MONITORING IS REQUIRED	
NIVOLUMAB	
-	Carcinoma of the esophagus
-	Adjuvant melanoma
-	Hodgkin's lymphoma (cHL)
-	Head and neck squamous cell carcinoma (HN SCC)
-	Renal carcinoma (RCC)
-	Non-small cell lung carcinoma (NSCLC)
-	Metastatic melanoma
PEMBROLIZUMAB	
-	Colorectal carcinoma (CRC)
-	Renal carcinoma (RCC)
-	Head and neck squamous cell carcinoma (HN SCC)
-	Hodgkin's lymphoma (cHL)
-	Urothelial carcinoma
-	Adjuvant melanoma
-	Non-small cell lung carcinoma (NSCLC)
-	Metastatic melanoma
ATEZOLIZUMAB	
-	Hepatocarcinoma (HCC)
-	Breast carcinoma
-	Small cell lung carcinoma (ES-SCLC)
-	Non-small cell lung carcinoma (NSCLC)

Table 1.5.3.1 List of tumor types treated with ICIs requiring AIFA monitoring.

For each antibody, indications for NSCLC, involving additional monitoring, have been summarized in *Table 1.5.3.2*. As in the case of atezolizumab, for the treatment of the same type of tumor, there may be more than one monitoring register, which takes into account different therapeutic approaches.

AIFA-MONITORED INDICATIONS OF ICIs FOR NSCLC
<p>PEMBROLIZUMAB (from 25/06/2017)</p> <ul style="list-style-type: none"> • In monotherapy, in the treatment of locally advanced and metastatic NSCLC, whose tumor expresses PD-L1 with tumor proportion score (TPS) >1%, in patients who have received at least one previous chemotherapy treatment. • In monotherapy in the first-line treatment of locally advanced and metastatic NSCLC whose tumor expresses PD-L1 with TPS > 50%. • In combination with pemetrexed and platinum-containing chemotherapy, in the first-line treatment of metastatic non-squamous NSCLC in adults whose tumor is not positive for EGFR or ALK. • In combination with carboplatin and paclitaxel or nab-paclitaxel, for the first-line treatment of metastatic squamous NSCLC.
<p>NIVOLUMAB</p> <ul style="list-style-type: none"> • In monotherapy for the treatment of locally advanced or metastatic NSCLC after prior chemotherapy in adults (from 25/03/2016). • In combination with ipilimumab and 2 cycles of platinum-based chemotherapy is indicated for the first-line treatment of metastatic NSCLC in adults with PD-L1 expression <50% (from 01/06/2022).
<p>ATEZOLIZUMAB</p> <ul style="list-style-type: none"> • In monotherapy for the treatment of locally advanced or metastatic NSCLC previously receiving chemotherapy (from 15/07/2018). • In monotherapy for the first-line treatment of adult patients with metastatic NSCLC whose tumors exhibit >50% PD-L1 expression on tumor-infiltrating immune cells (from 01/06/2022).

Table 1.5.3.2 List of ICIs use indications for NSCLC that require monitoring by AIFA.

In the ICIs' monitoring registers there are common exclusion criteria which, if present, constrain prescription. These features, that patients must not have, are related to the mechanism of action of the antibodies that interact with the immune system. For all 3 antibodies, affirmation to these rulings blocks the prescribing process:

- Previous therapy with anti PD1, anti PD-L1

- Pneumonia or interstitial lung disease
- Autoimmune diseases in active phase
- Treatment with systemic immunosuppressive drugs (except low-dose <10 mg/day of corticosteroids; low-dose corticosteroids for adrenocortical insufficiency; inhaled mineralocorticoids) 2 weeks prior to treatment.

In addition to these exclusion criteria, the remaining characteristics, that a patient must possess in order to receive treatment, excluding age >18 years, differ from antibody to antibody and from line of therapy used. Generally, the patient must first receive an anatomic-pathological assessment of the tumor mass to determine the right prescription for these antibodies. The analysis of cytomorphological characteristics must establish a histology different than small cell and specify the NSCLC subtype. The biopsy results also must consider EGFR mutations, ALK rearrangements, and PD-L1 expression. It must also be established that the patient has metastases. And the oncologist, before proceeding with treatment, must assess the patient's general condition, which, as explained in section 1.3.1, is defined by clinical examination through the ECOG performance status.

The characteristics that must be examined to verify treatment, extracted from the monitoring sheets of the AIFA registries, are reported below for each antibody and summarized in the following tables.

1.5.3.1 Eligibility criteria of pembrolizumab

The Figure 1.5.3.1.1 summarizes all eligibility criteria for first-line pembrolizumab distinguishing between combination and monotherapy. The criterion that constrains the choice of monotherapy or combination is PD-L1 expression less than or greater than 50%. According to histology, both the choice of antineoplastic agents and the reporting required for EGFR and ALK driver mutations vary as shown in Figure 1.5.3.1.1.

According to the criteria of the monitoring registries, the following characteristics should be verified to define an appropriate prescription of first-line pembrolizumab:

- NSCLC
- Metastasis (TMN staging = IV)
- PFS \leq 2
- PD-L1 assessed and quantifiable
- Histology (squamous cell carcinoma, adenosquamous adenocarcinoma, large cell carcinoma, NOS carcinoma)

- EGFR and ALK negative or not performed

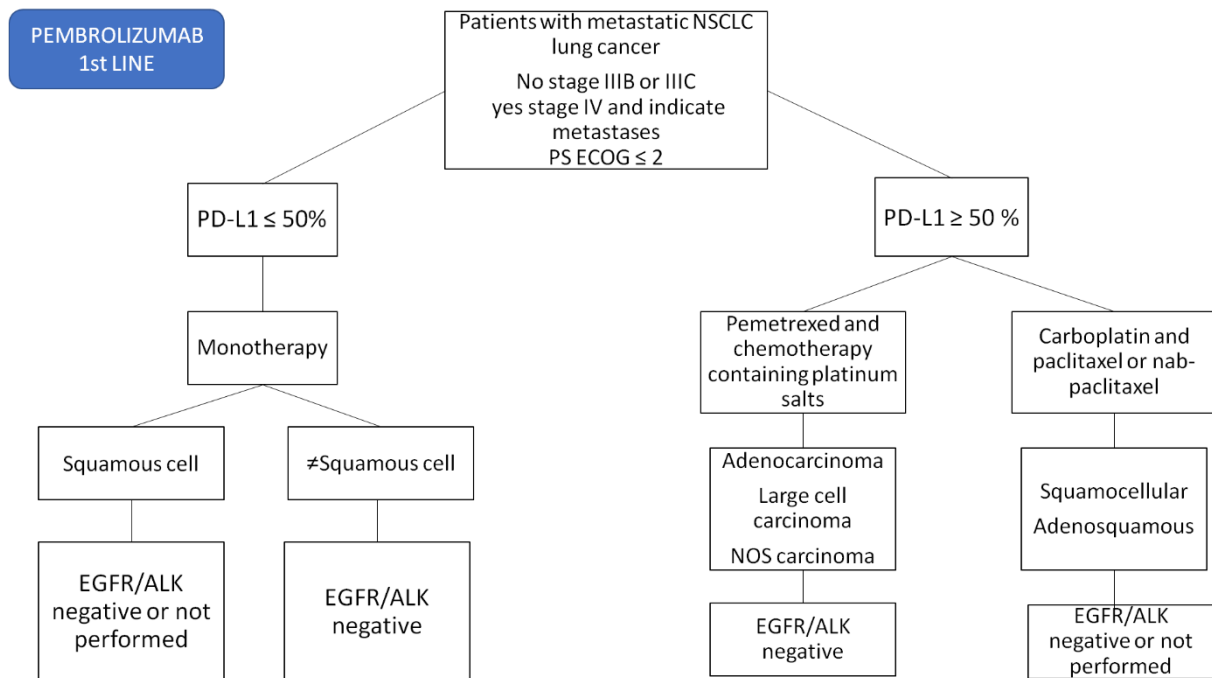


Figure 1.5.3.1.1 Schematic representation of the first-line treatment approaches of pembrolizumab according to eligibility criteria.

Instead criteria to be verified for pembrolizumab 2nd/3rd line are:

- NSCLC
- Metastasis (TMN staging = IIIB, IIIC, IV)
- Presence of other previous therapy (chemotherapy or anti EGFR/ALK if 3rd line)
- PS ≤ 2
- PD-L1 assessed and quantifiable
- EGFR and ALK negative or not performed (2nd line only)

Unlike the first-line prescription does not require specific PD-L1 levels; it is sufficient that PD-L1 is assessable. The required assessments of EGFR and ALK mutations vary by line of therapy as seen in Figure 1.5.3.1.2.

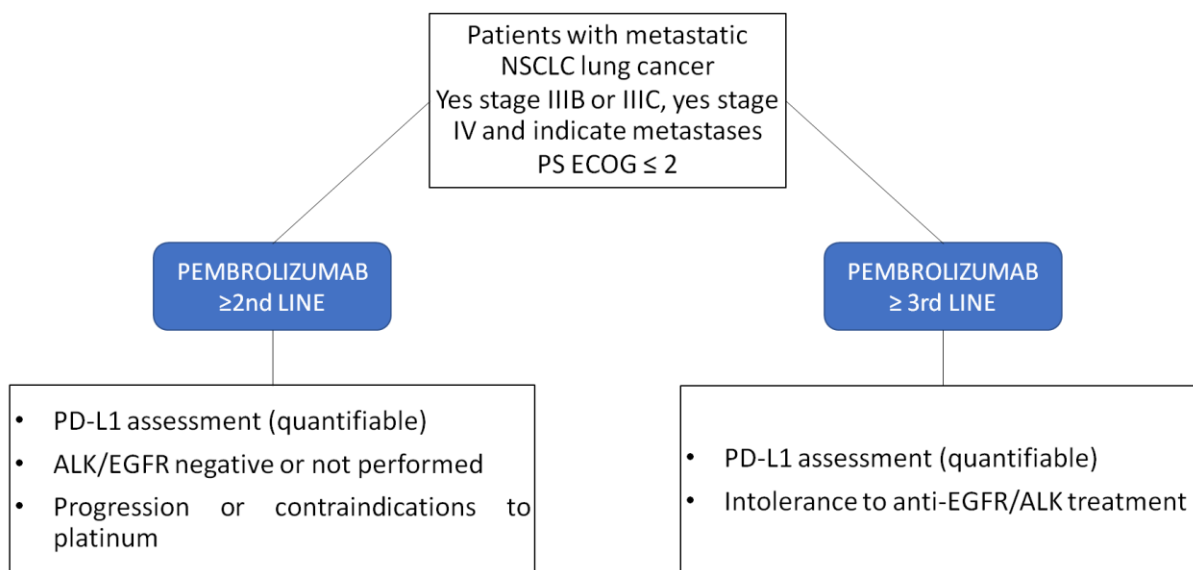


Figure 1.5.3.1.2 Schematic representation of following lines of pembrolizumab treatment strategies according to eligibility requirements.

1.5.3.3 Eligibility criteria of nivolumab

Indications under AIFA monitoring include the use of nivolumab as monotherapy or, from June 2022, in combination with Ipilimumab. In the study, patients received only treatment in monotherapy. In monotherapy, the selection criteria relate to ECOG performance status and histology as reported in Figure 1.5.3.2.1.

Criteria to be verified for nivolumab 2nd line monotherapy:

- NSCLC
- Advanced squamocellular NSCLC (TMN staging = IIIB, IV)
- Presence of another previous therapy (chemotherapy)
- PS ≤ 2

Criteria to be verified for nivolumab 2nd line association:

- NSCLC
- Metastasis (TMN staging = IV)
- Presence of other previous therapy (chemotherapy or anti EGFR/ALK if 3rd line) not for metastases
- PS ≤ 2
- PD-L1 assessed and quantifiable
- EGFR and ALK negative

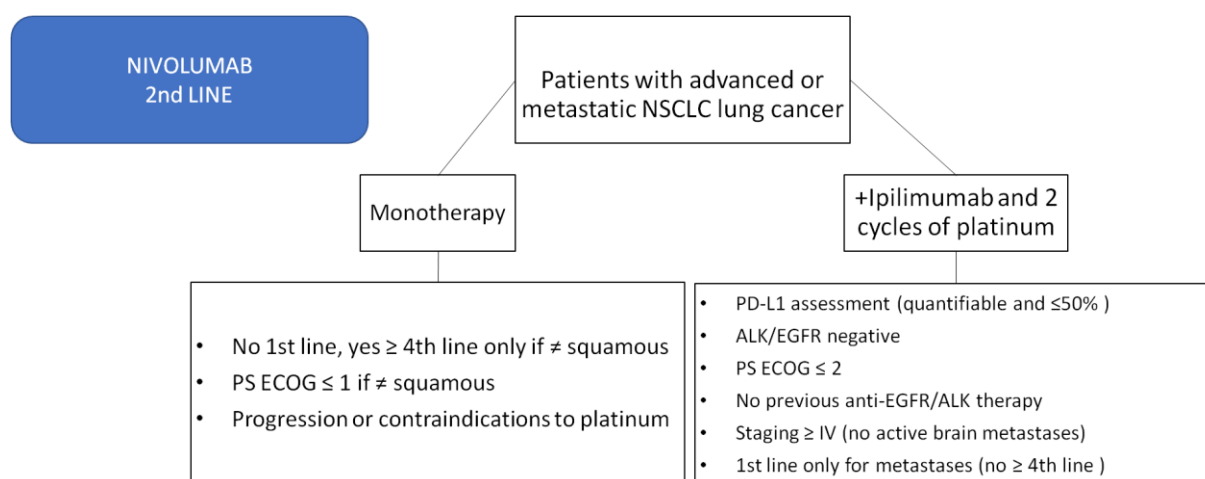


Figure 1.5.3.2.1 Schematic representation of the second-line treatment approaches of nivolumab according to eligibility criteria.

1.5.3.3 Eligibility criteria of atezolizumab

Atezolizumab is indicated in the AIFA registries as both first-line and second-line. Patients in the study received only the second-line because the first-line indication, whose selection criteria are shown in Figure 1.5.3.3.1, was introduced from June 2022.

As described in Figure 1.5.3.3.2, the second- and subsequent-line indication, such as nivolumab, does not require specific PD-L1 levels and involves different assessments of driver mutations depending on the line of therapy.

Criteria to be verified for atezolizumab 1st line

- NSCLC
- Metastasis (TMN staging = IV)
- PS ≤ 2
- PD-L1 assessed and quantifiable (on CT ≥ 50%, on CI ≥ 10 %)
- EGFR and ALK negative (not performed only if squamous cell)

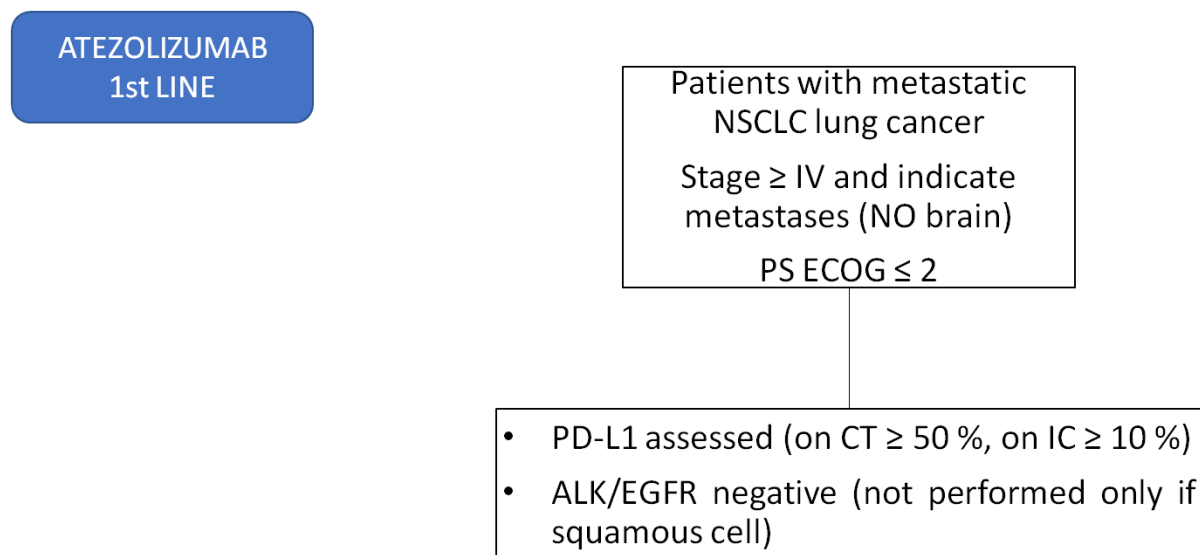


Figure 1.5.3.3.1 Schematic representation of the first-line treatment approaches of atezolizumab according to eligibility criteria.

Criteria to be verified for atezolizumab 2nd-3rd line

- NSCLC
- Metastasis or advanced (TMN staging = IIIB, IV)
- PS ≤ 2
- Presence of other previous therapy (chemotherapy or anti EGRF/ALK if 3rd line)
- EGFR and ALK negative or not performed (positive from 3rd line onwards)

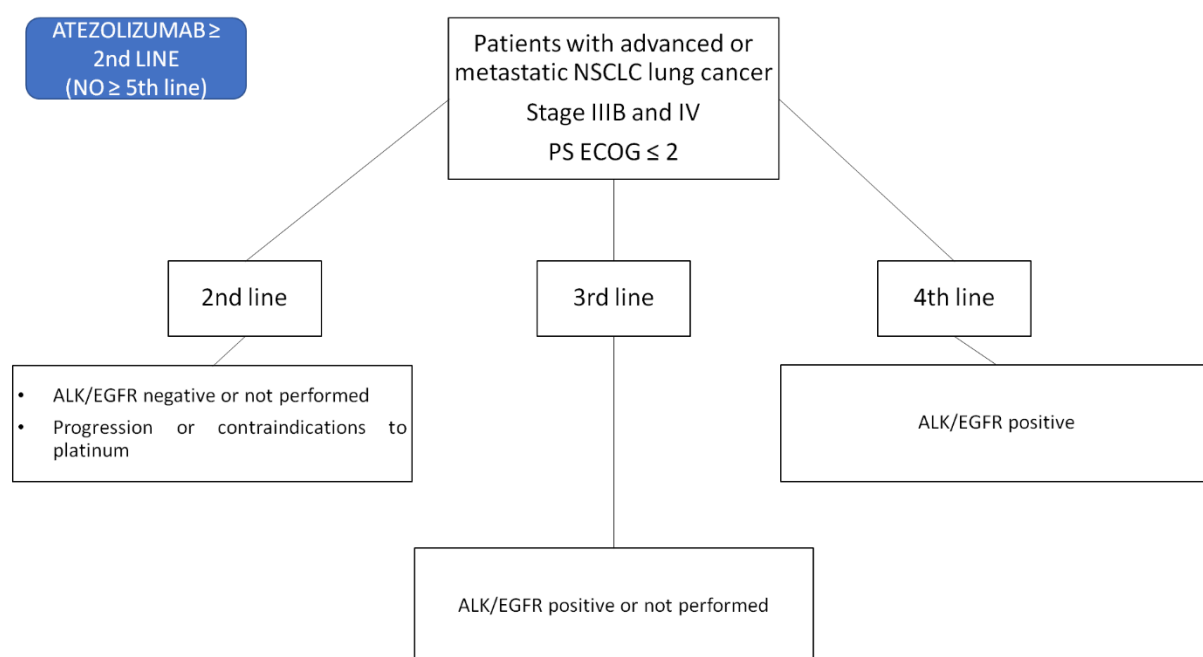


Figure 1.5.3.3.2 Schematic representation of following lines of atezolizumab treatment strategies according to eligibility requirements.

1.5.4 Pembrolizumab

Pembrolizumab, brand name Keytruda, is a humanized monoclonal anti PD-1 (programmed cell death-1) antibody produced in Chinese hamster ovary cells by recombinant DNA technology.⁶¹

It has been on the market in hospital or specialised structures since 10/04/2017 as 50 mg powder for concentrate for solution for infusion.

Each vial of powder contains 100 mg pembrolizumab. After reconstitution, a 4 ml vial 25 mg/ml is obtained.

Must be stored between 2-8°C, away from light, do not freeze, after opening use immediately.

Clear to slightly opalescent, colourless to slightly yellow solution, pH 5.2 – 5.8.

It is produced by Merck Sharp & Dohme Limited.

There are two dosage schedules 200 mg every 3 weeks or 400 mg every 6 weeks as monotherapy OR 200 mg every 3 weeks in combination, both administered as a 30-minute infusion.

Pembrolizumab is still covered by unexpired patent protection with recognised therapeutic innovativeness at a high level of attention.

Indications for pembrolizumab (Indications for 11 types of tumors)

- Melanoma
- Non-small cell lung carcinoma (NSCLC)
- Classical Hodgkin's lymphoma (cHL)
- Urothelial carcinoma
- Head and neck squamous cell carcinoma (HNSCC)
- Renal cell carcinoma (RCC)
- Carcinomas with high microsatellite instability (MSI-H, microsatellite instability-high) or mismatch repair deficient (dMMR, mismatch repair deficient)
- Carcinoma of the esophagus
- Triple negative breast carcinoma (TNBC)
- Endometrial carcinoma (EC)
- Carcinoma of the cervix

Patients should be treated with KEYTRUDA until disease progression or unacceptable toxicity (and up to maximum duration of therapy if specified for an indication). Atypical responses (i.e.

an initial transient increase in tumor size or small new lesions within the first few months followed by tumor shrinkage) have been observed.⁶¹ It is recommended to continue treatment for clinically stable patients with initial evidence of disease progression until disease progression is confirmed.⁶¹

1.5.4.1 Pivotal trials of pembrolizumab

Pembrolizumab demonstrated efficacy in KEYNOTE-024, a phase III randomized trial comparing single agent pembrolizumab against platinum chemotherapy in untreated stage IV NSCLC patients. In this trial, patients with tumors expressing PD-L1 tumor proportion score (TPS) $\geq 50\%$ demonstrated superior response rate of pembrolizumab monotherapy over chemotherapy, 44.8% vs. 27.8%, and superior overall survival, median OS 30.0 months (95% CI 18.3 months–not reached) vs. 14.2 months (95% CI 9.8 vs. 19.0 months)⁶². The overall survival benefit of pembrolizumab monotherapy was also demonstrated in patients with PD-L1 TPS of $\geq 1\%$ in KEYNOTE-042, a randomized phase III trial which demonstrated superior overall survival in untreated metastatic NSCLC patients receiving pembrolizumab compared to chemotherapy in patients with TPS $\geq 50\%$, TPS $\geq 20\%$, and TPS $\geq 1\%$ ⁶³. In the exploratory analysis, overall survival of pembrolizumab was not statistically significant in patients with TPS 1%–49%, which suggested that survival benefit in TPS $\geq 1\%$ group was primarily driven by improved survival in patients with TPS $\geq 50\%$ ⁶³.

1.5.5 Nivolumab

Nivolumab monoclonal anti-PD-1 antibody, is produced in Chinese hamster ovary cells by recombinant DNA technology.⁶⁴

It has been on the market in hospital or specialised structures since 28/10/2015 as concentrate for solution for infusion in which each mL of concentrate contains 0.1 mmol (or 2.5 mg) of sodium.

OPDIVO 10 mg/mL is marketed as: 4 mL vial containing 40 mg nivolumab, 10 mL vial containing 100 mg nivolumab and 24 mL vial containing 240 mg nivolumab.

It appears like a clear to opalescent, colourless to pale yellow liquid that may contain few light particles. The solution has a pH of approximately 6.0 and an osmolality of approximately 340 mOsm/kg.

The recommended dose of OPDIVO, depending on the indication, is either 240 mg nivolumab every 2 weeks with a 30-minute infusion or 480 mg every 4 weeks with a 60-minute infusion. It is manufactured by Bristol-Myers Squibb Pharma EEIG.

Nivolumab should be stored between 2-8°C, away from light, do not freeze, use immediately after opening.

This high-cost oncology drug, with recognised therapeutic innovativeness, is still covered by unexpired patent protection.

Treatment with OPDIVO, either as a monotherapy or in combination with ipilimumab or other therapeutic agents, should be continued as long as clinical benefit is observed or until treatment is no longer tolerated by the patient (and up to maximum duration of therapy if specified for an indication).⁶⁴ For adjuvant therapy, the maximum treatment duration with OPDIVO is 12 months.⁶⁴

For OPDIVO in combination with cabozantinib, OPDIVO should be continued until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression.⁶⁴ Atypical responses (i.e., an initial transient increase in tumor size or small new lesions within the first few months followed by tumor shrinkage) have been observed.⁶⁴ It is recommended to continue treatment with nivolumab or nivolumab in combination with ipilimumab for clinically stable patients with initial evidence of disease progression until disease progression is confirmed.⁶⁴ Dose escalation or reduction is not recommended for OPDIVO as monotherapy or in combination with other therapeutic agents. Dosing delay or discontinuation may be required based on individual safety and tolerability.⁶⁴

Indications of nivolumab: (Indications for 12 types of tumors)

- Melanoma
- Adjuvant treatment of melanoma
- Non-small cell lung carcinoma (NSCLC)
- Malignant mesothelioma of the pleura (MPM)
- Renal cell carcinoma (RCC)
- Classical Hodgkin's lymphoma (cHL)
- Squamous cell carcinoma of the head and neck (SCCHN)
- Urothelial carcinoma
- Colorectal carcinoma (CRC) with mismatch repair deficiency (dMMR) or high microsatellite instability (MSI-H)
- Esophageal squamous histotype carcinoma (OSCC)

- Adjuvant treatment of oesophageal or gastro-oesophageal junction cancer (OC or GEJC)
- Adenocarcinoma of the stomach, gastro-oesophageal junction (GEJ) or esophagus

1.5.5.1 Pivotal trials of nivolumab

The efficacy of nivolumab as monotherapy was evaluated in two open-label RCTs comparing nivolumab vs docetaxel in adult patients with squamous (CheckMate017 study⁶⁵) or non-squamous histology (CheckMate057 study⁶⁶), progressed during or after platinum-based chemotherapy and with ECOG PS 0-1. In both studies, subjects were included regardless of PD-L1 expression. The majority of patients (80%) were stage IV and had ECOG PS 1, only a minority were aged ≥ 75 years. In the CheckMate017 study, patients with previous systemic treatment for metastatic disease were not included, whereas in the CheckMate057 study they were included, although they constituted a minority of the sample (12%). Determination of mutational status for the EGFR, ALK and KRAS genes was only performed in the CheckMate057 study with confirmed positivity in 14%, 4%, 11% of cases, respectively. In both trials, patients were randomised 1:1 to receive nivolumab (3mg/Kg every two weeks) or Docetaxel (75 mg/m² every three weeks) until unacceptable toxicity or progression. Nivolumab statistically significantly increased OS vs DOCE in both patients with squamous histology (+3.2 months) and those with non-squamous histology (+2.8 months) with a 41% and 27% reduction in the risk of death, respectively⁶⁵⁻⁶⁶. The efficacy results were confirmed at a subsequent 2-year follow-up⁶⁷. Quality of life analysis showed that nivolumab improved disease-related symptoms and general health status compared to Docetaxel for second-line treatment of advanced non-squamous NSCLC⁶⁸. In patients with squamous histology, nivolumab relieved the burden of symptoms and improved health status compared to Docetaxel⁶⁹. In both phase III studies, the final primary analysis was matched to the pre-planned interim analysis. This may lead to an overestimation of the effect (risk of bias in both studies).

Although the nivolumab-ipilimumab combination is indicated in the treatment of NSCLC, it was not considered in the study due to its inclusion in the monitoring registry as of 01/06/2022.

1.5.6 Atezolizumab

Atezolizumab is an Fc-engineered, humanized IgG1 anti-programmed death-ligand 1 (PD-L1) monoclonal antibody produced in Chinese hamster ovary cells by recombinant DNA technology⁷⁰.

It has been on the market in hospital or specialized structures since 24/01/2018 as 840 mg and 1.200 mg concentrate for solution for infusion.

One 14 mL vial of concentrate contains 840 mg of atezolizumab.

One 20 mL vial of concentrate contains 1 200 mg atezolizumab.

After dilution, the final concentration of the diluted solution should be between 3.2 and 16.8 mg/mL that appears like clear, colourless to slightly yellowish liquid.

The recommended dose of Tecentriq is either 840 mg administered intravenously every two weeks, or 1200 mg administered intravenously every three weeks, or 1 680 mg administered intravenously every four weeks.

The initial dose of Tecentriq must be administered over 60 minutes. If the first infusion is well tolerated, all subsequent infusions may be administered over 30 minutes.

Dose reductions of Tecentriq are not recommended.

It is manufactured by Roche SpA.

Nivolumab should be stored between 2-8°C, away from light, do not freeze, use immediately after opening.

This high-cost oncology drug, not recognised as innovative drugs, is still covered by unexpired patent protection

Indications for atezolizumab (Indications for 5 types of tumors)

- Urothelial carcinoma
- Non-small cell lung carcinoma
- Small cell lung carcinoma
- Triple-negative breast carcinoma
- Hepatocellular carcinoma

Induction phase: four to six cycles.

Maintenance phase: until disease progression or onset of unmanageable toxicity.

Atypical responses (initial disease progression followed by reduction of tumor mass) have been observed in association with continued treatment with Tecentriq after disease progression. Treatment after disease progression may be considered at the discretion of the physician⁷⁰.

1.5.6.1 Pivotal trials of atezolizumab

The efficacy of Atezolizumab, in lines after the first one, was evaluated in the open-label phase III RCT OAK⁷¹⁻⁷²⁻⁷³, in which adult patients with squamous (about 26%) or non-squamous (about 74%) stage IIIB/IV NSCLC with ECOG 0-1 performance status were enrolled. Subjects

were included independently from the expression grade of PD-L1. Enrolled patients received at least two previous lines of chemotherapy. The co-primary endpoints were OS in the ITT population and OS in the subpopulation with PD-L1 levels $\geq 1\%$ in tumor cells or tumor-infiltrating immune cells. Initially, 850 patients were enrolled (primary efficacy population ITT850). Subsequently, the total number of patients increased to 1225 (secondary efficacy population ITT1225) in order to allow a comparison analysis in patients with high PD-L1 expression levels. Atezolizumab statistically significantly increased OS, both in the ITT850 population (+4.2 months) and in the population with PD-L1 levels $\geq 1\%$ (+5.4 months), reducing the risk of death by 25% and 26%, respectively. No PFS advantage was obtained in these analyses. Subgroup investigations according to PD-L1 expression levels show that the efficacy of atezolizumab is particularly higher in the composite subgroup of patients expressing the highest PD-L1 levels. In such patients the absolute OS benefit was 10, 8 months with a 55% reduction in the risk of death⁷³. In these patients, the PFS benefit was very weak.

The Table 1.5.1 provides a summary of the three antibodies' key properties.

Brand name	KEYTRUDA®	OPDIVO®	TECENRIQ®
Active ingredient	Pembrolizumab	Nivolumab	Atezolizumab
Producer	Merck Sharp & Dohme Limited	Bristol-Myers Squibb	Roche
ATC	L01XC18	L01XC17	L01XC17
Pharmaceutical form	Powder for concentrate for infusion	Concentrate for infusion	Concentrate for infusion
Dose	100 mg	40, 100, 240 mg	1.200 mg
Posology	200 mg q3w, 400 mg q6w (monotherapy only); iv; up to unacceptable progression or toxicity	240 mg q2w, 480 mg q4w; iv; up to loss of clinical benefit or unacceptable toxicity	1.200 mg q3v; iv; up to loss of clinical benefit or unacceptable toxicity
Therapeutic category	Antineoplastic drugs - monoclonal antibodies	Antineoplastic drugs - monoclonal antibodies	Antineoplastic drugs - monoclonal antibodies

Therapeutic innovation	<ul style="list-style-type: none"> - Metastatic NSCLC 1st line monotherapy (innovation validity period from 25/06/2017 until 24/06/2020) - Metastatic NSCLC 1st line monotherapy (innovation validity period from 25/06/2017 until 24/06/2020) -Metastatic non-squamous NSCLC 1st line in association (innovation validity period from 11/12/2019 until 10/12/2022) -Metastatic squamous NSCLC 1st line in association (innovation validity period from 17/12/2020 until 16/12/2023) 	Locally advanced or metastatic NSCLC 2nd line monotherapy (validity period from 22/02/2017 until 21/02/2020)	Locally advanced or metastatic NSCLC 2nd line monotherapy
Classification for reimbursability purposes	H - centres designated by the region	H - centres designated by the region	H - centres designated by the region
Classification for delivery purposes	Drug subject to a restrictive medical prescription for use exclusively in hospital or a similar structures	Drug subject to a restrictive medical prescription for use exclusively in hospital or a similar structures	Drug subject to a restrictive medical prescription for use exclusively in hospital or a similar structures
Negotiating conditions	Direct SSN discount, to structures; payback discount to regions	Direct SSN discount, to structures; payback discount to regions	Direct SSN discount, to structures

Table 1.5.1 Summary of the characteristics of the three antibodies

1.6 Real life studies

The urgency of ensuring access to cancer treatment in the shortest amount of time makes it critical to assess the efficacy and cost-effectiveness of therapy in a concrete and objective way. For this reason regulatory agencies and scientific societies have recently emphasized the significance of real-world clinical and administrative health data collection to enhance scientific evidence about the safety and efficacy of medical therapies ⁷⁴.

The U.S. Food And drug Administration has encouraged the gathering of real data in post-marketing drug monitoring, regulation, and approval processes⁷⁵. Randomized clinical trials frequently lack external validity because they usually include selected patients who account for 2% to 4% of the overall cancer population; indeed, these trials are under-representative of some patient categories, such as elderly patients or patients with poor performance status, who are eligible for treatment in clinical practice⁷⁶. These critical issues might be solved by real-world studies, in which data collection from medical records reflects the experience of most patients with cancer. In this context, electronic health data sets are useful to be matched with study data in order to map all patients treated with a specific drug. On the other hand, administrative data are usually anonymized; thus they cannot capture the safety and effectiveness of a specific therapeutic pathway or deep biological and genomic data. These two data sources may complement each other in order to collect quality, complete, and reliable data, thus improving scientific evidence from randomized trials in a modern drug development model⁷⁷.

1.7 Aim

The monoclonal antibodies pembrolizumab, nivolumab and atezolizumab, considering their multiple indications of use, represent the highest expenditure items on the direct purchases of ULSS 3. Analyzing the costs and consumption of these drugs, it was necessary to describe in the real clinical practice context of NSCLC, the treatment modalities and clinical outcomes observed with the antibodies. Therefore the aim of the study was to compare the clinical outcome of ULSS 3 Serenissima patients with the pivotal studies of the ICI, by checking the presence of the eligibility conditions that allows a prescription to be defined as appropriate.

Unlike to "mature" medicines, which have a variety of real world evidence, oncological and onco-haematological drugs still have a paucity of data that may be used to plan and manage pharmaceutical care and spending. The selection of patients who are eligible for expensive medications, the evaluation of the prescription's appropriateness by comparing the use of the medication in clinical practice with the therapeutic recommendations (an analysis of the over- and under-use), and, finally, the calculation of value for money (the investment made in terms of clinical and financial needs for the healthcare system), should all be considered standard control measures. To ensure that all people have access to adequate treatment, the assessment of pharmaceutical products should be viewed as an essential aspect for a weighted allocation of resources in a system with limited resources, such as the Italian National Health System. Real world study became a tool to evaluate prescriptive appropriateness of these therapies, in both

clinical and economic terms, and to highlight areas of over and under use defining the correct place in therapy. Through real world evidence, it is also possible to highlight the critical issues of the diagnostic path of lung cancer to improve analysis of tumor characterization and consequently the choice of the correct therapy.

2. MATERIALS AND METHODS

The project conducted in ULSS 3 Serenissima is an observational, retrospective, single-centre study performed using real world data from administrative databases, the onco-haematological therapy management systems and the AIFA Monitoring Registers.

The materials used coincide with data obtained from the ULSS 3 population of 625,189 patients. The data on patients, the four oncology departments, and drugs administered enabled the writing of this paper.

The study is divided into three sub-studies analyzing the appropriateness and clinical efficacy of the following drugs: Pembrolizumab, nivolumab, atezolizumab in the hospital districts of Chioggia, Mestre, Venice, Dolo and Mirano.

For pembrolizumab and nivolumab the observation period starts on 01/01/2018 and ends on 24/07/2022, while for atezolizumab from 01/01/2019 to 24/07/2022. The study dates vary depending on the times when the antibodies were first employed in ULSS 3, which also reflects the dates of their introduction into the market and their inclusion in the monitoring registry.

Although the marketing dates for pembrolizumab and nivolumab are prior to the study start period, the choice of the starting dates was constrained by the absence of data, due to the lack of informatic registers before 2018.

2.1 Data Management

Data for this study were extracted from:

- The Aifa Monitoring Registers, activated for the ULSS 3 Prescribing Centres, provided information on the patient's histology, line of therapy, evaluation of EGFR mutation, ALK rearrangements, PD-L1 expression, tumor staging and ECOG performance status.
- The administrative database of ULSS 3 Serenissima:
 - Assisted Patient Registry used to collect the patients' personal information (tax code, first name, surname, date of birth, sex and ULSS 3 district of residence)
 - Regional registry of deceased persons updated to 30/06/2022: this allowed the identification of the death dates
 - FAR-OSP database contains anonymously information on drugs used within the hospital. FAR-OSP constitutes an information flow for monitoring the consumption of medicines in hospitals, and is designed to collect data on medicines used in healthcare facilities directly managed by the National Health Service, with the exception of medicines dispensed by them in direct distribution.

- DDF3 database, including two channels of drug distribution (Direct Distribution and Ambulatory Care), allowed to analyze the expenditure and consumption of antibodies in the study. DDF database provides information on pharmaceutical services supplied on an ambulatory setting, as well as on drugs dispensed through direct distribution and DPC. Unlike to FAR-OSP, where data on patients who received treatment are not reported, DDF allows the identification of consumption per patient. For this reason, the FAR-OSP database was used only for the exploratory analysis of costs and consumption, in order to complete the missing data of the DDF, which only reports ambulatory use of some high-cost oncology drugs.
- The pathological anatomy database containing histological and molecular tumor reports from 2016 to 2022. It was constructed by data extraction from the anatomical-pathology management systems of the various districts (Mestre, Venice, Chioggia, Dolo-Mirano).
- The onco-haematology therapy management system B-MIND is a platform that manages the entire lifecycle of antineoplastic drugs, from clinical prescription to administration. B-MIND is used in ULSS 3 for planning the set-up phases of infusion oncology therapies. It allowed the selection of patients treated for NSCLC and to obtain administration information such as infusion date and dosage.

2.2 Drug selection

In ULSS 3 Serenissima, the pharmaceutical categories with the most impacts, according to ATC, may be found through the examination of spending in 2021.

The FAR-OSP and DDF3 regional flows, fed by ULSS companies, provided the basis for the analysis, by identifying the drugs to be studied.

The 2021 data from these two databases were merged together in order to have an overall view of the annual expenditure, excluding from the DDF3 database the drugs ambulatory distribution, already present in the FAR-OSP database.

The Anatomical Therapeutic Chemical classification system (ATC), used for the systematic classification of drugs and supervised by the World Health Organisation, was essential for the analysis. By dividing the pharmaceutical categories by ATC (listed in Table 2.2.1), it was possible to frame the amount of expenditure on oncology drugs. The analysis conducted on Microsoft Access grouped drugs with ATCs starting with L0* (antineoplastic and immunomodulatory drugs) and compared them with all other drugs with different ATCs. Then, the spending of the different anatomical groupings was sorted by decreasing amount. Finally,

the total amount of the various therapy groups was calculated, it represents the total sum of direct and ambulatory expenditure.

ATC
A= GASTROINTESTINAL SYSTEM AND METABOLISM
B= BLOOD AND HAEMOPOIETIC ORGANS
C= CARDIOVASCULAR SYSTEM
D= DERMATOLOGICAL SYSTEM
G= GENITO-URINARY SYSTEM AND SEX HORMONES
H= SYSTEMIC HORMONAL PREPARATIONS, EXCL. SEX HORMONES AND INSULINS
J= GENERAL ANTIMICROBIALS FOR SYSTEMIC USE
L= ANTINEOPLASTIC AND IMMUNOMODULATORY DRUGS
M= MUSCULOSKELETAL SYSTEM
N= NERVOUS SYSTEM
P= PESTICIDES, INSECTICIDES AND REPELLENTS
R= RESPIRATORY SYSTEM
S= SENSE ORGANS
V= VARIOUS

Table 2.2.1 The first level of ATC subdivision contains the main anatomical group.

The investigation was then restricted to medications with ATCs equivalent to L0* (reported in Table 2.2.2) to compare costs across the several therapeutic subgroups. In this case, the various ATCs were grouped by filtering down to the third level. To find the subgroup that had the greatest impact on spending, the total for each subgroup was sorted in descending order.

ATC	THERAPEUTIC GROUP
L01A*	ALKYLATING AGENTS
L01B*	ANTIMETABOLITES
L01C*	PLANT ALKALOIDS AND OTHER NATURAL PRODUCTS
L01D*	CYTOTOXIC ANTIBIOTICS AND RELATED SUBSTANCES
L01E*	PROTEIN KINASE INHIBITORS
L01X*	OTHER ANTINEOPLASTIC AGENTS
L02A*	HORMONE AND RELATED AGENTS
L02B*	HORMONE ANTAGONISTS AND RELATED AGENTS
L03A*	IMMUNOSTIMULANTS
L04A*	IMMUNOSUPPRESSANTS

Table 2.2.2 The third level of ATC subdivision contains the pharmacological therapeutic subgroup.

Then the costs of the related chemical subgroups of the first three therapeutic subgroups with the highest economic impact (L01E* = protein kinase inhibitors, L01X* = other antineoplastic

agents, L04A* = immunosuppressants) were analyzed. The ATC explosion reached the fourth level (Tables 2.2.3; 2.2.4; 2.2.5), to understand which chemical subgroups presented the greatest impact.

L04A*	IMMUNOSUPPRESSANTS
L04AA*	Selective immunosuppressants
L04AB*	Tumor necrosis factor alpha (TNF- α) inhibitors
L04AC*	Interleukin inhibitors
L04AD*	Calcineurin inhibitors
L04AE*	Other immunosuppressants

Table 2.2.3 Classification immunosuppressant pharmacological therapeutic subgroup.

L01X *	OTHER ANTINEOPLASTIC AGENTS
L01XA*	Platinum compounds
L01XB*	Methylhydrazines
L01XC*	Monoclonal antibodies
L01XD*	Sensitizers used in photodynamic/radiation therapy
L01XF*	Retinoids for cancer treatment
L01XG*	Proteasome inhibitors
L01XK*	Poly (ADP-ribose) polymerase (PARP) inhibitors
L01XY*	Combinations of antineoplastic agents

Table 2.2.4 Classification other antineoplastic agents pharmacological therapeutic subgroup detected in the analysis.

L01E*	PROTEIN KINASE INHIBITORS
L01EA*	BCR-ABL tyrosine kinase inhibitors
L01EL*	Bruton's tyrosine kinase (BTK) inhibitors
L01EX*	Other protein kinase inhibitors
L01EF*	Cyclin-dependent kinase (CDK) inhibitors
L01EB*	Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors
L01EJ*	Janus-associated kinase (JAK) inhibitors
L01EC*	B-Raf serine-threonine kinase (BRAF) inhibitors
L01ED*	Anaplastic lymphoma kinase (ALK) inhibitors
L01EE*	Mitogen-activated protein kinase (MEK) inhibitors
L01EG*	Mammalian target of rapamycin (mTOR) kinase inhibitors

Table 2.2.5 Classification protein kinase inhibitors pharmacological therapeutic subgroup detected in the analysis.

Among the various chemical-therapeutic subgroups, the subgroup of monoclonal antibodies L01XC* (belonging to the therapeutic subgroup of the other antineoplastic agents L01X* and reported in Table 2.2.6) was the one that most impacted on expenditure. For this reason it was considered appropriate to select this group of drugs as the subject of study.

It was investigated which types of antibodies weighed more on spending, finding that pembrolizumab, nivolumab and atezolizumab presented the highest costs to be incurred. It was necessary to assess consumption and prescribing patterns by evaluating the clinical outcomes in accordance with the presence or absence of AIFA requirements for treatment.

MONOCLONAL ANTIBODIES
DARATUMUMAB
PEMBROLIZUMAB
NIVOLUMAB
RITUXIMAB
PERTUZUMAB
TRASTUZUMAB
ATEZOLIZUMAB
TRASTUZUMAB EMTANSINE
CETUXIMAB
DURVALUMAB
BLINATUMOMAB
OBINUTUZUMAB
INOTUZUMAB OZOGAMICINA
AVELUMAB
ISATUXIMAB

Table 2.2.6 Monoclonal antibodies list of other antineoplastic agents pharmacological therapeutic subgroup.

2.2.1 Comparison of spending on immunomodulatory and antineoplastic drug in the years 2021, 2020, and 2019

Using the regional FAR-OSP and DDF3 databases, all antineoplastic and immunomodulatory drugs (with ATC= L0*) were put in order of price, from the highest to the least expensive, taking into consideration the total annual expenditure for each active ingredient. Using the regional FAR-OSP and DDF3 databases, all antineoplastic and immunomodulatory drugs (with ATC= L0*) were ranked in order of price, from the highest to the least expensive, taking into consideration the total annual expenditure for each active ingredient. This was useful to check if in the years prior to 2021 the drugs investigated also showed the same trends in utilization and spending.

2.3 Selection of the pathology

To perform an initial skimming of the different tumor forms to be investigated in the study, for each monoclonal antibody, the summary of product characteristics sheet, found on the AIFA website, was referenced to observe the individual therapeutic indications for each specialty.

To further narrow the field of study, it was planned to select as tumor types, only those treatable with the antibodies under investigation that have indications reimbursable by the NHS.

Therefore, lung cancer was chosen because nowadays it remains a high mortality neoplasm, which globally represents the first cause of cancer mortality in the male population and the third cause of mortality in the female gender. Lung cancer is identified as a high-incidence disease: for the male population the second cancer in terms of incidence and the third cancer in terms of incidence in the female population.

NSCLC was then chosen, because unlike SCLC, it has more complex therapeutic management and more treatment choices.

2.4 Cohort selection

For the selection of the cohort, the starting database was the computerized B-MIND management system of ULSS 3 Serenissima, containing data on all infusion therapies set up in ULSS 3.

The procedures that allowed the cohort to be formed were all performed using Microsoft Excel software.

To go to identify the number of patients with lung cancer treated with immunotherapy, a database was created containing all the infusion therapies set up in ULSS 3, by merging the extractions of the Mestre-Venice, Chioggia, and Dolo-Mirano management systems.

In the realized database, thanks to the presence of the field named "Diagnosis," it was possible to know for each therapy what type of tumor it had been set up for. So by filtering by "polm" the field of diagnoses, it was possible to obtain the list of all patients who were treated for lung cancer in 2021 (including both primary and secondary lung cancers).

Then using the "active pinciple" field, only therapies related to the antibodies under study were isolated.

Leaving out therapies classified as cytotoxic, only therapeutic schemes containing monoclonal antibodies were filtered out (Table 2.4.1):

ATEZOLIZUMAB
ATEZOLIZUMAB + CARBOPLATIN +ETOPOSIDE
NIVOLUMAB
PEMBROLIZUMAB + CARBOPLATIN + PACLITAXEL
PEMBROLIZUMAB + CARBOPLATIN + PACLITAXEL + ALBUMINA
PEMBROLIZUMAB + CARBOPLATIN + PEMETREXED
PEMBROLIZUMAB + CISPLATINO + PEMETREXED
PEMBROLIZUMAB + CARBOPLATIN + TAXOL
PEMBROLIZUMAB + PEMETREXED

Table 2.4.1 Therapeutic schemes containing monoclonal antibodies.

By using excel, a pivot table was created to divided patients according to the active ingredient administered.

Then from the AIFA registries, for each active ingredient, all data of the patients who took the ICIs were manually extracted. Patient tax codes obtained from AIFA and patient tax codes extracted from B-MIND were cross-checked using ACCESS. The comparison obtained allowed the exclusion of patients who were included wrongly from the b-mind management system.

Nine patients drawn from B-MIND were excluded either due to diagnoses that were found in AIFA to be other than NSCLC (such as diagnosis of SCLC or Carcinoma of the Head and Neck) or due to having received treatments other than immunotherapy (ex. pemetrexed). At the same time, patients in AIFA who showed a monitoring registry activation in the year 2017 were excluded.

The AIFA registry was used only as a confirming tool, while the B-MIND management system played a key role in structuring the project. Unlike AIFA, B-MIND reports only those therapies that were effective set up. The B-MIND database was also helpful to check the correspondence between the therapies actually set up and those in the AIFA registries. Clearly, the reliability of the b-mind database is not absolute either. Despite the rarity of cases, it is possible that set up therapy due to poor health status does not take it. Another problem concerns the lack of data related to a delayed computerization of onco-hematology therapy set-up in the first years of the management system's introduction. In the extractions transmitted from Dolo and Mirano related to the year 2018, a gap of tax codes could be observed, representing the patients who according to AIFA had received treatment.

Despite these issues, the reference database with a structural role for court formation was B-MIND.

Respectively, 83 patients treated with pembrolizumab (77 first-line and 6 second-line), 52 with nivolumab, and 42 with atezolizumab were selected.

2.4.1 Inclusion Criteria

Incident patients who received at least one treatment with pembrolizumab, nivolumab, or atezolizumab for the treatment of non-small cell lung cancer from 2018 (2019 for atezolizumab) to 2020, according to AIFA and B-mind management, were included.

2.4.2 Exclusion Criteria

Patients who did not appear in both databases (B-MIND and AIFA registries), who started treatment at times other than study periods, and who did not have a primary diagnosis of NSCLC were excluded.

2.5 Antibody databases

For each antibody, a database including the information below was made:

- patients' personal data (first name, last name, tax code, sex, date of birth, hospital district of residence). The patients' data were already present in the databases extracted from B-MIND but in order to verify their correctness, they were then cross-checked with the ULSS 3 assisted registry.
- date of death obtained from the deceased registry (updated to 30/06/2022)
- pharmaceutical data of the different oncology active ingredients, including both therapies set up for infusion administration extracted from b-mind from 2018 to 2022, and oral oncology therapies dispensed in direct distribution, extracted from DDF3 including the years 2017 to 2021.

For each active ingredient administered, the date of the first administration and the date of the last administration were reported. The difference between these two dates allowed calculation of the duration of therapy, identifying the treatment period expressed in days. In case of patient death, the time period between the first dose and the day of death "days_decease" and the time period between the last dose and the date of death "days_deceasef" was calculated. Additional information of the active ingredients administered were age at first administration, dosage received, number of administrations and description of the schedule.

-data manually extracted from AIFA registries, reporting: histologic, line of therapy, evaluation of EGFR mutations and ALK rearrangements, stage of disease, start and end date of therapy,

number of administrations received, performance status, metastasis, motivation for end of treatment, exam used to rule the end of treatment.

- pathology anatomy data: all the texts of the reports, for each fiscal code performed by patients from 2016 to 2022, were reported within a single cell. Anatomy-pathological examinations performed in the years antecedent to the study period were also taken into account to exclude additional tumor sites and to avoid missing reports of those patients, already in the second or third line, who started treatment for lung cancer long before immunotherapy administrations. All database structuring processes and subsequent methods used were the same for each antibody. In the case of different analysis processes in the three substudies, differences will be emphasized.

2.5.1 Stratification of patients

For comparison of clinical outcomes the patients treated with the same ICI were divided according to line of therapy and according to the absence or presence of associations with other drugs. For comparison of clinical outcomes the patients treated with the same ICI were divided according to line of therapy and according to the absence or presence of associations with other drugs.

By summarizing the information for each active ingredient on a single line, it was possible both to distinguish whether the 'immunotherapy in question was first- or second-line, and to identify the presence of any other active ingredients administered at the same time thus decreeing the administration of monotherapy or combination therapy.

As already anticipated data for drugs administered by intravenous infusion were extracted from B-MIND using extractions from 2018 to 2022, data for oral therapies were extracted from the 2017, 2018, 2019, 2020, 2021 databases of DDF3.

The identification of oral therapies was conducted by merging all DDF3 databases from the years 2017 to 2022, from which all drugs included in account distribution or dispensed in ambulatory settings were excluded. The tax codes of the ICIs patients were cross-matched with those of the DDF3, isolating for each patient all active ingredients that were dispensed through direct distribution.

Of these oral therapies, only those indicated in cases of oncogene addicted metastatic disease, i.e., all tyrosine kinase inhibitors used for the treatment of lung cancer patients with the presence of driver mutations, were filtered through the ATC field.

The ATCs selected for research of oral therapies are shown in the table 3.5.1.1.

ATC	DESCRIZIONE ATC
L01EB02	ERLOTINIB
L01EB01	GEFITINIB
L01EB03	AFATINIB
L01EB04	OSIMERTINIB
L01ED01	CRIZOTINIB
L01ED03	ALECTINIB
L01ED04	BRIGATINIB
L01ED05	LORLATINIB
L01EX14	ENTRECTINIB
L01ED01	CRIZOTINIB
L01EX12	LAROTRECTINIB
L01EC02	DABRAFENIB
L01EE01	TRAMETINIB

Table 3.5.1.1 Tyrosine kinase inhibitors list of researched in the DDF3 database.

To make the stratification process faster, through SAS software, an algorithm (reported in Table 3.5.1.2) was devised to automatically define whether ICIs were administered as monotherapy and as first-line treatment.

To define monotherapy, in cases where the date of attack of the immunotherapy was subsequent, and thus major, to the date of first administration of active ingredient X, it was verified that the last administration of active ingredient X was antecedent to the date of first administration of the antibody. Or, in cases where the date of first administration of the active ingredient was later than the date of attack of the immunotherapy, it was verified that the date of end of immunotherapy treatment was also major than the date of start of active ingredient X. If in either case the conditions coexisted then the value 1= "monotherapy" was returned, otherwise the value 0= "association". For each patient, a "monotherapy" field was filled out with these two values.

The first line has been established just observing whether the start date of immunotherapy treatment was earlier and therefore lower than that of active ingredient X. Again, if the condition coexisted the value 1= "first line" was returned, otherwise 0= "second line". A "first line" field was entered for each patient in which these two values were indicated.

To get further confirmation of progression, the presence of a second treatment was confirmed checking whether the start date of active ingredient X was greater than the date of the antibody first administration. If the condition was verified, the value 1= "second treatment" was returned, otherwise it has been assigned 0= "immunotherapy last treatment".

Monotherapy/Assotiation

```
(dtfinal_IMMUNO<=dtinitial1 or dtfinal1<=dtinitial_IMMUNO) and  
(dtfinal_IMMUNO<=dtinitial2 or dtfinal2<=dtinitial_IMMUNO) and  
(dtfinal_IMMUNO<=dtinitial3 or dtfinal3<=dtinitial_IMMUNO) and  
(dtfinal_IMMUNO<=dtinitial4 or dtfinal4<=dtinitial_IMMUNO) and  
(dtfinal_IMMUNO<=dtinitialDDF or dtfinalDDF<=dtinitial_IMMUNO)  
then  
monotherapy=1;  
else  
monotherapy=0;
```

First or second line

```
if dtinitial_IMMUNO>dtinitial1 and  
   dtinitial_IMMUNO>dtinitial2 and  
   dtinitial_IMMUNO>dtinitial3 and  
   dtinitial_IMMUNO>dtinitial4 and  
   dtinitial_IMMUNO>dtinitialDDF then  
second_line=1;  
else  
second_line=0;
```

Second treatment

```
if dtfinal_IMMUNO<dtinitial1 or  
   dtfinal_IMMUNO<dtinitial2 or  
   dtfinal_IMMUNO<dtinitial3 or  
   dtfinal_IMMUNO<dtinitial4 or  
   dtfinal_IMMUNO<dtinitialDDF then  
progression=1;  
else  
progression=0;
```

Table 3.5.1.2 Representation of the algorithm used to define first-line, monotherapy, and further treatment after immunotherapy.

2.5.2 Evaluation of prescriptive appropriateness

By matching the tax codes of study patients with those in the anatomic pathology database, it was possible to compare the data in the anatomic pathology reports with those within the AIFA registries.

The comparison allowed us to mark the eligibility criteria extracted from AIFA as:

“WRONG”: if the AIFA data had opposite characteristics to those found in the pathology anatomy reports.

“NOT CONFIRMED”: if no information related to that specific characterization was found in the pathology anatomy reports.

“NOT COMPARABLE”: if the patient in the pathology anatomy database of the entire ULSS 3 from 2016 to 2022 had no reports.

“NOT EVALUATED”: patients who in both AIFA and Pathology Anatomy reports present no information regarding Histology, gene mutational status, or PD-L1 expression.

“CORRECT”: patients who present matching information in both AIFA and pathology reports.

The sum of patients with that particular incorrect criterion was made for each eligibility criteria reported on AIFA (histological, EGFR, ALK, PD-L1). Criteria defined as incorrect were those classified as "wrong" and "not confirmed".

All patients with data defined as incorrect were summed, and the relative percentage over the total number of patients was calculated.

For each type of incorrect eligibility criteria, both the percent of patients who went on to progression and the percent of patients who went on to death, were reported over the totality of patients presenting the incorrect criteria.

Inappropriate prescriptions were defined as those that contained incorrect eligibility criteria. To demonstrate that the choice of therapy based on incorrect criteria led to adverse therapeutic outcomes (progression and death), curves were created for each antibody by stratifying patients according to different eligibility criteria. For each eligibility criteria, the OS and PFS of patients with defined incorrect criteria were compared with those of patients presenting "not comparable," "not evaluable," and "correct" eligibility criteria.

After that, the appropriateness analysis focused on pembrolizumab.

It was verified that patients had received the right treatment schedule, monotherapy or combination with chemotherapeutics, according to PD-L1 expression. Inappropriate cases were summed up and the error rate was calculated on the total number of pembrolizumab study.

For patients receiving pembrolizumab in association, it was verified that the correct chemotherapy regimen according to tumor histology had been associated. It was verified that patients with squamous histology, as determined by AIFA registries, had received a doublet of carboplatin and paclitaxel, and it was verified that patients with non-squamous histology had pemetrexed.

In the AIFA registries, the lack of evaluation of EGFR mutations and ALK rearrangements in patients with squamous histology causes the blocking of the prescribing process. It was checked how many of these patients, from the anatomic pathology database, had a negative mutational assessment.

In the AIFA registries, the lack of evaluation of EGFR mutations and ALK rearrangements in patients with squamous histology causes the blocking of the prescribing process. It was checked how many of these patients, from the anatomic pathology database, had a negative mutational assessment. Patients with an incorrect mutational assessment, i.e., other than negative, were summed for both EGFR and ALK. The error rate on the total number of patients with squamous histology was calculated for both ALK and EGFR.

The focus shifted to second lines of immunotherapy, where it was verified that patients treated with nivolumab and atezolizumab presented systemic treatment before immunotherapy. Through the "first line" field, it was verified that all patients presented the value 0, meaning second line.

2.5.3 PFS (Progression Free Survival) and OS (Overall Survival) curve generation

To define whether a patient was deceased or not, tax codes obtained from pharmaceutical databases were matched with those from the death registry. For deceased patients, the date of death was reported. Through excel, all patients reporting a date of death were assigned a code = 1, however, if the patient had no date of death in the "death" field, the value 0 was assigned. on the other hand, for progression was created a proxy in which the end date of treatment was assumed to correspond with disease progression.

However, using the AIFA data of end-of-treatment motivation, those cases that presented "toxicity" "clinical decision" "partial response" or "stable" as the end-of-treatment motivation and at the same time did not present a date of death were excluded.

As to indicate death, also for progression was created a named column, reporting the values: 1 = progression, 0 = no progression.

For pembrolizumab, survivorship curves were performed both on the totality of patients and by isolating patients who received pembrolizumab as first-line treatment.

The paucity of second-line patients did not allow the development of a curve for this subpopulation. Atezolizumab and Nivolumab curves were generated and subsequently compared with each other, as these two drugs are both recommended for the treatment of patients previously treated with chemotherapy.

Different survival curves were derived for each antibody stratified according to:

- histologic, where survival was compared between patients with adenocarcinoma, squamous carcinoma and NOS;
- performance status ECOG where survival was compared between patients with PS ECOG=0, 1 and 2;
- PD-L1 where patients with PD-L1 expression $\leq 1\%$, $\geq 1\% \leq 49\%$, $\geq 50\%$ were compared.

All curves and survival values, with 95% confidence interval, were calculated by R software, using the survival library.

The times used to calculate the curves were converted from days to months by dividing the number of days by 30.417.

2.6 Statistical analysis

The data produced from the analyses performed during the study were obtained by directly analysing the entire population defined during the selection of the cohorts. The database management software used for the selection and the extraction of the data from the databases analysed were Microsoft Access 2016 and SAS Data Management for the databases that exceed the computing capacity of Microsoft Access. The analyses of the raw data extracted using the database management software were performed using a spreadsheet in Microsoft Excel 2016 and the graphic representation of the data obtained was done using Microsoft PowerPoint 2016. The Kaplan–Meier method was used to estimate progression-free and overall survival. Rstudio was utilized to calculate survival curves.

The clinical outcomes of the study used to compare real-life treatment performance with that reported in the registrational studies were:

- PFS= median progression free survival
- OS = median overall survival
- 6 months survival rate
- 6 months progression rate

- ORR objective response rate
- DOR duration of response

All progression and survival data were extrapolated from the curves.

ORR was calculated as the percentage of patients who had no progression, no date of death, and who reported "clinical decision" "partial response" in the AIFA end-of-treatment motivations.

DOR was calculated as the averaged time to response. Time to response was defined as the difference between the date of end and start of immunotherapy treatment and was converted from days to months by dividing by 30.417.

The clinical outcomes extrapolated from the real-world studies of ULSS 3 were then compared with those from the registrational studies.

To compare clinical outcomes, populations were first compared according to age, sex, histological, ALK rearrangement positivity, EGFR mutation positivity, and PS ECOG.

The different dosages used, the study duration and follow-up period, and the average number of administrations were compared.

3. RESULTS

3.1 Pharmaceutical spending analysis

ATC	Expenditure
TOTAL	€ 131.386.019,81
L= ANTINEOPLASTIC AND IMMUNOMODULATORY DRUGS	€ 55.385.289,67
J= GENERAL ANTIMICROBIALS FOR SYSTEMIC USE	€ 21.042.554,72
B= BLOOD AND HAEMOPOIETIC ORGANS	€ 20.953.082,59
A= GASTROINTESTINAL SYSTEM AND METABOLISM	€ 10.012.116,39
N= NERVOUS SYSTEM	€ 5.037.660,71
V= VARIOUS	€ 4.315.833,17
H= SYSTEMIC HORMONAL PREPARATIONS, EXCL.SEX HORMONES AND INSULINS	€ 3.125.491,04
M= MUSCULOSKELETAL SYSTEM	€ 3.046.310,96
C= CARDIOVASCULAR SYSTEM	€ 2.823.584,13
R= RESPIRATORY SYSTEM	€ 2.036.202,05
S= SENSE ORGANS	€ 1.639.455,91
D= DERMATOLOGICAL SYSTEM	€ 981.913,34
G= GENITO-URINARY SYSTEM AND SEX HORMONES	€ 813.170,86
P= PESTICIDES, INSECTICIDES AND REPELLENTS	€ 32.245,56

Table 3.1 Total expenditure of 2021 concerning direct purchases and drugs administered in ambulatory settings subdivided by ATC.

From the analysis of pharmaceutical spending in 2021 according to ATC classification (of which the methods of analysis were explained in Chapter 3.1), it could be seen that antineoplastic and immunomodulatory drugs represented 42% of the total expenditure of 2021 concerning direct purchases and drugs administered in ambulatory settings. As can be seen from the Table 3.1, onco-hematology drugs represent the first item of expenditure with an annual cost of about 55 million euros.

ANTINEOPLASTIC AND IMMUNOMODULATORY DRUGS	Expenditure
IMMUNOSUPPRESSANTS	€ 20.099.170,30
OTHER ANTINEOPLASTIC AGENTS	€ 15.836.538,37
PROTEIN KINASE INHIBITORS	€ 12.128.374,35
HORMONE ANTAGONISTS AND RELATED AGENTS	€ 2.581.505,56
ANTIMETABOLITES	€ 1.478.281,96
IMMUNOSTIMULANTS	€ 1.247.730,15
HORMONE AND RELATED AGENTS	€ 1.091.857,15
PLANT ALKALOIDS AND OTHER NATURAL PRODUCTS	€ 731.343,57
CYTOTOXIC ANTIBIOTICS AND RELATED SUBSTANCES	€ 198.374,55
ALKYLATING AGENTS	€ 158.747,93

Table 3.2 2021 expenditure of antineoplastic and immunomodulatory drugs anatomic group.

Among all antineoplastic and immunomodulatory drugs, drugs with ATC equal to L01X* (other antineoplastic agents) represented the second most expensive expenditure item as shown in the Table 3.2.

OTHER ANTINEOPLASTIC AGENTS (L01X)	Expenditure
Monoclonal antibodies	€ 13.039.863,79
Proteasome inhibitors	€ 1.534.455,29
VEGF/VEGFR (Vascular Endothelial Growth Factor) inhibitors	€ 930.761,98
Combinations of antineoplastic agents	€ 164.904,48
Poly (ADP-ribose) polymerase (PARP) inhibitors	€ 73.048,80
Platinum compounds	€ 56.344,62
Retinoids for cancer treatment	€ 26.327,60
Sensitizers used in photodynamic/radiation therapy	€ 8.526,88
Methylhydrazines	€ 2.304,92

Table 3.3 2021 expenditure of therapeutic group L01X (other antineoplastic agents).

As highlighted in the Table 3.3, the therapeutic chemical group of monoclonal antibodies accounts for 82% of spending of all "other antineoplastic agents" and almost 10% of total pharmaceutical spending (ambulatory and direct).

Table 3.4 2021 expenditure of therapeutic chemical group L01XC (monoclonal antibodies).*

MONOCLONAL ANTIBODIES	Expenditure
DARATUMUMAB	€ 3.118.318,71
PEMBROLIZUMAB	€ 2.610.104,57
NIVOLUMAB	€ 2.152.665,35
RITUXIMAB	€ 1.416.867,34
PERTUZUMAB	€ 860.021,05
TRASTUZUMAB	€ 745.287,55
ATEZOLIZUMAB	€ 636.949,20
TRASTUZUMAB EMTANSINE	€ 603.854,89
CETUXIMAB	€ 590.745,71
DURVALUMAB	€ 168.065,82
BLINATUMOMAB	€ 62.346,48
OBINUTUZUMAB	€ 18.255,20
INOTUZUMAB OZOGAMICINA	€ 48.325,20
AVELUMAB	€ 7.935,72
ISATUXIMAB	€ 121,00

The antibodies under investigation represent relatively the second, third and seventh largest expenditure items of all monoclonal antibodies as reported in Table 3.4. The sum of spending on these antibodies amounts to 3.050.629,12 euros: representing 2.32% of total annual pharmaceutical spending.

ATC	Desc. ATC	Expense 2021	Expense2020	Expense 2019	Δ 2021-2020	Δ 2021-2019	Δ% 2021-2020	Δ% 2021-2019
L04AX04	LENALIDOMIDE	€ 5.313.027,39	€ 5.594.101,01	€ 4.735.352,62	-€ 281.073,62	€ 577.674,77	-5%	12%
L01XC24	DARATUMUMAB	€ 3.118.318,71	€ 2.704.143,77	€ 1.759.587,08	€ 414.174,94	€ 1.358.731,63	15%	77%
L01XC18	PEMBROLIZUMAB	€ 2.610.104,57	€ 2.100.653,93	€ 1.373.287,50	€ 509.450,64	€ 1.236.817,07	24%	90%
L01XC17	NIVOLUMAB	€ 2.152.665,35	€ 2.151.475,30	€ 2.838.042,34	€ 1.190,05	-€ 685.376,99	0%	-24%
L01EL01	IBRUTINIB	€ 2.032.915,29	€ 1.819.105,37	€ 1.517.964,54	€ 213.809,92	€ 514.950,76	12%	34%
L04AX07	DIMETILFUMARATO	€ 1.632.616,74	€ 1.453.116,76	€ 1.282.989,31	€ 179.499,98	€ 349.627,43	12%	27%
L02BB04	ENZALUTAMIDE	€ 1.507.704,04	€ 1.100.318,33	€ 906.144,42	€ 407.385,71	€ 601.559,61	37%	66%
L01XC02	RITUXIMAB	€ 1.416.867,34	€ 1.221.277,19	€ 1.283.200,45	€ 195.590,15	€ 133.666,89	16%	10%
L01EB04	OSIMERTINIB	€ 1.405.145,11	€ 863.997,72	€ 193.743,16	€ 541.147,39	€ 1.211.401,96	63%	625%
L04AA06	ACIDO MICO FENOLICO	€ 1.277.099,39	€ 1.045.038,65	€ 1.082.958,48	€ 232.060,74	€ 194.140,91	22%	18%
L04AA25	ECULIZUMAB	€ 1.097.476,92	€ 1.386.076,89	€ 1.178.333,90	-€ 288.599,97	-€ 80.856,98	-21%	-7%
L01EF01	PALBOCICLIB	€ 1.045.548,04	€ 1.328.569,30	€ 1.069.341,93	-€ 283.021,26	-€ 23.793,89	-21%	-2%
L04AA23	NATALIZUMAB	€ 983.181,13	€ 850.854,62	€ 598.995,54	€ 132.326,51	€ 384.185,59	16%	64%
L01XC13	PERTUZUMAB	€ 860.021,05	€ 1.028.384,40	€ 762.994,89	-€ 168.363,35	€ 97.026,16	-16%	13%
L04AA33	VEDOLIZUMAB	€ 830.766,37	€ 730.826,89	€ 679.284,17	€ 99.939,48	€ 151.482,20	14%	22%
L04AC08	CANAKINUMAB	€ 829.939,04	€ 838.719,70	€ 388.153,98	-€ 8.780,66	€ 441.785,07	-1%	114%
L02BX03	ABIRATERONE	€ 788.726,40	€ 901.060,16	€ 867.940,48	-€ 112.333,76	-€ 79.214,08	-12%	-9%
L01EJ01	RUXOLITINIB	€ 784.413,52	€ 835.466,88	€ 775.969,80	-€ 51.053,36	€ 8.443,72	-6%	1%
L04AA27	FINGOLIMOD	€ 784.144,92	€ 630.078,56	€ 551.702,96	€ 154.066,36	€ 232.441,95	24%	42%
L01EA03	NILOTINIB	€ 758.774,86	€ 851.392,19	€ 769.366,43	-€ 92.617,34	-€ 10.591,57	-11%	-1%
L01XC03	TRASTUZUMAB	€ 745.287,55	€ 819.884,13	€ 1.059.157,56	-€ 74.596,58	-€ 313.870,01	-9%	-30%
L02AE02	LEUPRORELINA	€ 699.117,51	€ 703.091,83	€ 741.039,05	-€ 3.974,32	-€ 41.921,54	-1%	-6%
L04AC05	USTEKINUMAB	€ 670.626,11	€ 686.161,85	€ 616.295,58	-€ 15.535,74	€ 54.330,53	-2%	9%
L04AB01	ETANERCEPT	€ 670.213,56	€ 584.329,28	€ 599.824,91	€ 85.884,28	€ 70.388,65	15%	12%
L04AX06	POMALIDOMIDE	€ 664.866,26	€ 490.494,80	€ 569.287,01	€ 174.371,46	€ 95.579,26	36%	17%
L01XC32	ATEZOLIZUMAB	€ 636.949,20	€ 736.625,78	€ 392.385,00	-€ 99.676,58	€ 244.564,20	-14%	62%

Table 3.5 Comparison of spending on immunomodulatory and antineoplastic drug in the years 2021, 2020, and 2019.

As can be seen in the Table 3.5, of all the drugs with ATC equal to L01 (antineoplastic and immunomodulatory), ICIs constitute the 3rd, 4th, and 26th items on the total expenditure.

For pembrolizumab there has been a large increase in spending both relative to 2020 and 2019. Nivolumab shows a decreasing trend relative to 2019. In contrast to atezolizumab which compared to 2019 shows an increasing expenditure.

3.2 Pembrolizumab outcomes

By cross-matching data from the B-MIND database and AIFA registries, 83 patients were selected who received pembrolizumab 200 mg every 3 weeks under treatment from 2018 to 2020.

As explained in Chapter 3.5.1 thanks to the algorithm implemented through SAS software, patients were stratified according to the first or second line of treatment and then were divided between monotherapy and combination.

FIRST LINE	77
MONOTHERAPY	53
ASSOCIATION	24
SECOND LINE	6
MONOTHERAPY	4
ASSOCIATION	2
TOT PEMBROLIZUMAB	83

Table 3.2.1 Stratification of patients receiving pembrolizumab.

As shown in Table 3.2.1, the majority of patients were treated as first-line (nearly 93%), while only 6 patients were treated as second-line.

Given the small number of second-line patients, the study focused on first-line patients, divided into 53 patients with pembrolizumab administration as monotherapy and 24 patients in combination. Due to the larger number of patients, comparisons were based more on monotherapy than combination.

All patients in the study with pembrolizumab had a diagnosis of stage IV metastatic NSCLC with a performance status (PS) between 0 and 2.

The maximum follow-up time of the patients was 53.81 months.

The median OS and PFS values were extrapolated for both totality of patients and first- and second-line patients. Subsequently, the median values of OS and PFS were also obtained for the patients on association and first-line monotherapy.

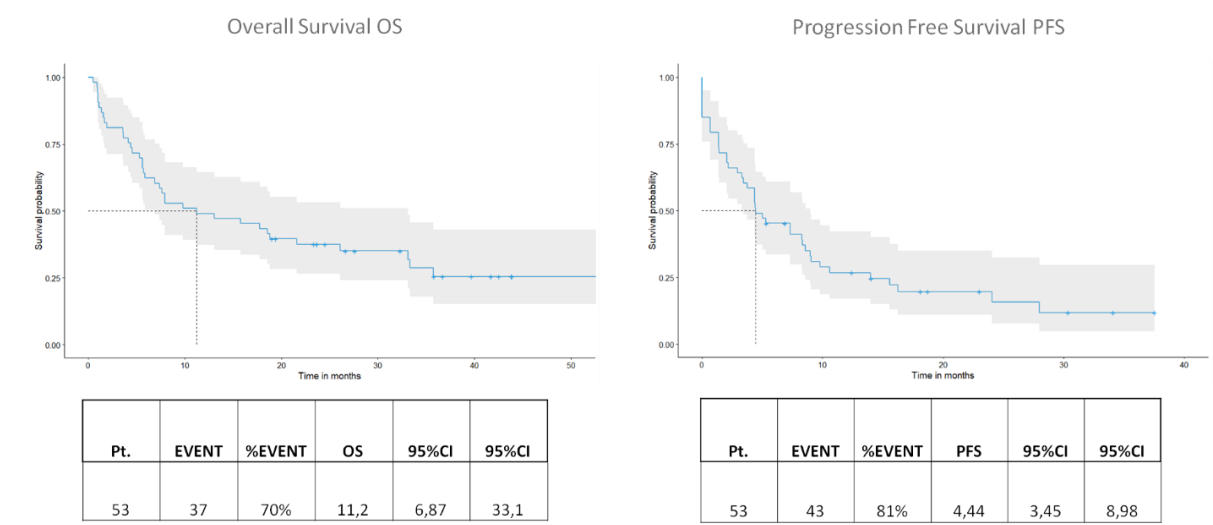


Figure 3.2.1 On the left the overall survival curve, on the right the progression-free survival curve of ULSS 3 patients treated with first-line pembrolizumab monotherapy.

Figure 3.2.1 shows the survival curves of the 53 patients on monotherapy compared with values extrapolated from the retrospective real-world efficacy study Cavaille et al.⁷⁸ and the registrational studies KEYNOTE024⁶² and KEYNOTE042⁶³. These studies verify the performance of pembrolizumab in first-line patients treated with monotherapy.

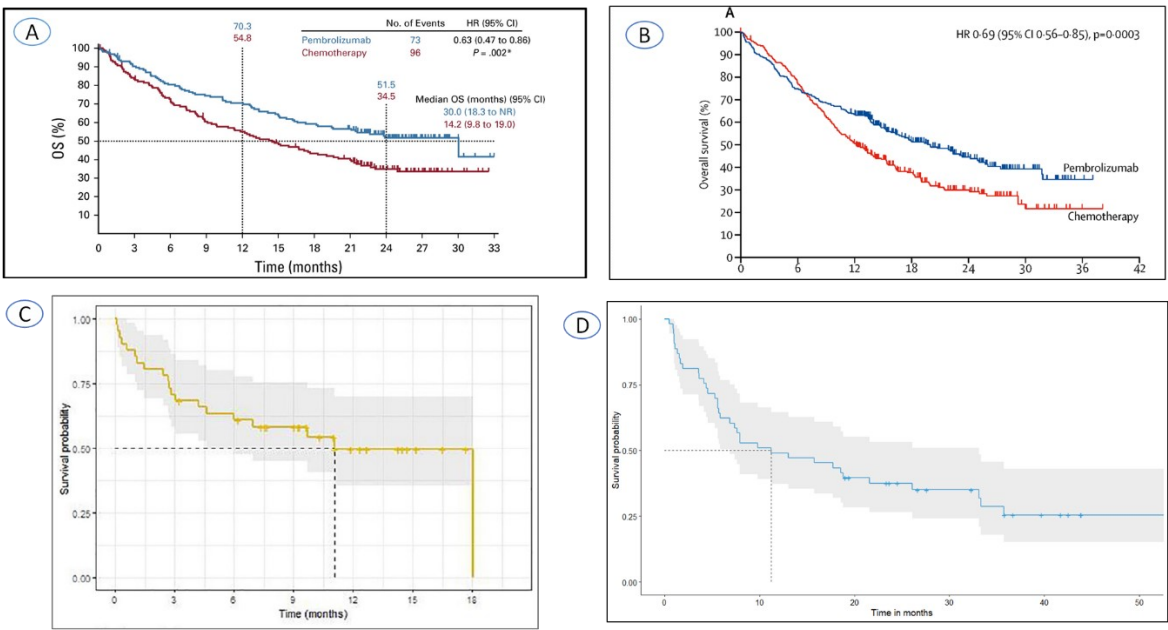


Figure 3.2.2 Kaplan-Meier estimates of overall survival: (A) population of updated KEYNOTE024; (B) PD-L1 TPS 50% or greater population of KEYNOTE042; (C) population

of real world study Cavaille et al, 2021 (D) population in first line monotherapy of ULSS 3 real world study.

Before comparing the different characteristics of the study population and the results obtained, we wanted to compare the similar pattern of survival curves shown in the Figure 3.2.2. In the KEYNOTE studies, where the efficacy of pembrolizumab was compared with chemotherapy, it can be seen that in the first few months of treatment, the red curve of pembrolizumab is underlying than that of chemotherapy. This trend is also seen in real-world studies (ULSS 3 and Cavaille et al.) where there is a rapid decrease in the early months of therapy to a stabilization in the long run.

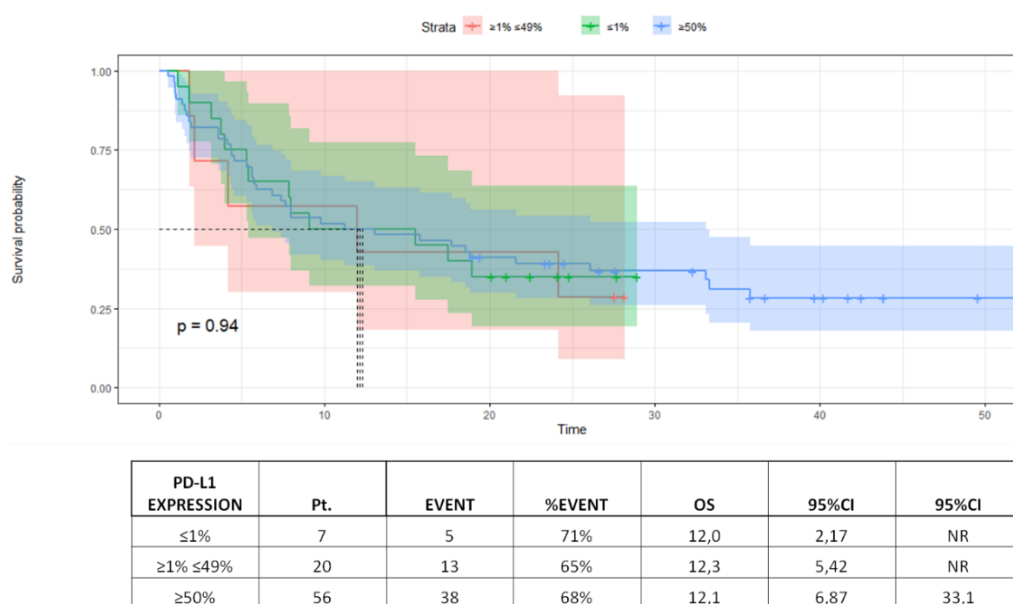


Figure 3.2.3 Pembrolizumab total population overall survival curve stratified by PD-L1 expression.

On the total population treated with pembrolizumab and the population treated with monotherapy different survival curves were calculated by stratifying according to histology, PS ECOG, according to PD-L1 expression to test whether the treatment could give better or worse outcomes relative to these variables. The curves generated resulted in no statistically significant difference. A nonsignificant example (p value 0.69) of stratification according to PD-L1 expression across the entire population treated with pembrolizumab is shown in Figure 3.2.3.

	REAL LIFE ULSS 3	REAL LIFE ULSS 3 (1L MONO)	REAL LIFE ULSS 3 (1L ASS.)	KEYNOTE 024 (1L MONO) ≥50%	KEYNOTE 042 (1L MONO) ≥50%	REAL LIFE 1L MONO
PATIENTS	83	53	24	154	299	41
MEDIAN AGE	69,1	69,1	69	64,5	63	64
RANGE	42-85	54-85	49-81	33-90	56-68	33-79
MALE	51 (61%)	37 (70%)	11 (46%)	92 (59,7%)	205 (69%)	20 (48,8%)
WOMEN	32 (39%)	16 (30%)	13 (54%)	62 (40,3%)	94 (31%)	21 (51,2%)
ECOG 0	21 (25%)	15 (28%)	5 (21%)	54 (35,1%)	96 (32%)	8 (19,5%)
ECOG 1	57 (69%)	34 (64%)	18 (75%)	99 (64,3%)	203 (68%)	22 (53,7%)
ECOG 2	5 (6%)	4 (5%)	1 (4%)	1 (0,6%)	0	6 (14,6%)
SQUAMOUS	18 (14%)	12 (23%)	1 (4%)	29 (18,8%)	107 (36%)	5 (12,2%)
NON SQUAMOUS	68 (82%)	39 (74%)	23 (96%)	125 (81,2%)	192 (64%)	36 (87,8%)
NOS	3 (4%)	2 (4%)	0	0	0	0
PD-L1 TPS ≤1%	7 (8%)	0	7 (29%)	0	0	0
PD-L1 TPS 1-49%	20 (24%)	1 (2%)	16 (67%)	0	0	0
PD-L1 TPS ≥50%	56 (67%)	52 (98%)	1 (4%)	154 (100%)	299 (100%)	41 (100%)
DEATHS	56 (67%)	37 (70%)	16 (67%)	7 (4,55%)	/	/
ALIVE	27 (31%)	6 (11%)	8 (33%)	147 (95,45%)	/	/
PROGRESSIVE DISEASE	77 (93%)	43 (81%)	22 (92%)	67 (43,50%)	23 (7,69%)	/
TREATMENT ONGOING	6 (7%)	10 (19%)	2 (8%)	23 (14,93%)	54 (18,06%)	/
OS	12,0 (7,63 - 24,2)	11,2 (6,87 - 33,1)	13,7 (5,42 - NR)	30 (18,3 - NR)	20,0 (15,4 - 24,9)	11,08 (5,98 - NR)
6m-Survival Rates	62,7% (5,31 - 7,40)	62,3% (50,5 - 76,8)	62,5% (45,8 - 85,2)	80,2% (72,9 - 85,7)	/	/
PFS	5,07 (3,5 - 8,4)	4,5 (3,5 - 9,1)	5,73 (2,87 - 13,3)	10,3 (6,7 - NR)	7,1 (5,9 - 9,0)	6 (3 - NR)
6m-Progression Rates	44,58% (45,8 - 85,2)	62,3% (50,5 - 76,8%)	62,5% (45,8% - 85,2)	62,1% (53 - 69,4)	/	/
ORR	7,22%	5,66%	8,33%	44,8% (36,8 - 53,0)	39% (29 - 38)	41,46%
DOR	8,59 (1 - 48,06)	8,54 (1 - 37,54)	8,68 (1 - 21,20)	3,9 (1 - 23,7)	10,8	/
Duration of observation	16,29 (1 - 53,81)	16,97 (1 - 53,81)	14,11 (1 - 28,92)	25,2 (20,4 - 33,7)	/	/
n° administration	11,93 (1 - 69)	11,55 (1 - 54)	5,34 (1 - 30)	10,5 (1-26)	/	9,49 (1-26)

Table 3.2.2 Summary of population characteristics and outcomes of the pembrolizumab study conducted in ULSS 3 and studies compared.

Below, the most significant differences between the study populations will be reported and the clinical outcomes of ULSS 3 patients treated as first-line monotherapy with pembrolizumab will be compared with the two registration studies KEYNOTE024⁶² and KEYNOTE042⁶³ and with a French retrospective real-life study by Cavaille et al.⁷⁸. All key data are summarized in the Table 3.2.2.

The ULSS 3 and Cavaille et al. real-life studies have similar sample sizes to each other, much lower than the KEYNOTE pivotal studies as seen in the Table 3.2.2.

The 53 patients from ULSS 3 treated with first-line pembrolizumab monotherapy had a mean age of 69.1 years. All studies used as comparisons have a lower mean age, despite the age range, particularly that of KEYNOTE024, being quite wide. Most (70 %) were men, data consistent with the pilot studies, and had an ECOG PS between 0 and 2 (only 5% had a PS =2). The percentage of patients with PS =2 in the registrational studies is much lower, in contrast to the Cavaille et al. real-life study, which has 14.6 % of patients.

In all studies, nonsquamous histology prevails over squamous.

No patients were found in ULSS 3 with EGFR mutations or positive ALK rearrangements.

In all compared studies monotherapy patients had PD-L1 >50%, in ULSS 3 only patient with PD-L1 less than 50% was found.

The percentages of patients in ULSS 3 who died and with progressing disease are much higher than those found in the KEYNOTE024 study.

The ULSS 3 median overall survival (OS) was 11.2 months (95% confidence interval [CI], 6,87-33,1). The ULSS 3 median progression free survival (PFS) was 4.5 months (95%

confidence interval [CI], 3,5 - 9,1). Both of these clinical outcomes are much lower than in the registry studies, they are, however, in line with the survival values found by Cavaille et al. in the real-life study.

The 6months-Survival Rates in ULSS 3 are also much lower than in the KEYNOTE024 study, while the 6months -Progression Rates are similar.

In the ULSS 3 Real life study patients were followed for a maximum period of 53.81 months (almost 4.5 years). The KEYNOTE024 and Cavaille et al. studies have maximum follow-up of 33.7 and 18 months, respectively, much shorter periods than the study conducted in ULSS 3.

Although in the registration studies Objective response rate (ORR) was calculated as the proportion of patients with a complete response or partial response to treatment according to Response Evaluation Criteria in Solid Tumors (RECIST), while in the ULSS 3 study ORR was calculated as the percentage of patients who had no progression, no date of death, and who reported "clinical decision" or "partial response" in the AIFA end-of-treatment motivations, the ORR found in ULSS 3 (5.66%) was much lower than in all other studies.

3.3 Nivolumab outcomes

By cross-matching data from the B-MIND database and AIFA registries, 52 patients who received at least one dose of nivolumab from 2018 to 2020 were selected. 13 patients received nivolumab 3 mg/kg every 2 weeks, only one patient has been treated with nivolumab 480 mg every 3 weeks and the majority (73%) was treated with nivolumab 480 mg every 3 weeks.

87% of patients received second-line treatment, while 7 patients received first-line treatment.

Indications monitored by AIFA, involve the use of nivolumab as monotherapy in pretreated patients with advanced or metastatic NSCLC, in fact all patients in the study with pembrolizumab had a diagnosis of NSCLC: 45 in metastatic stage (IV), 7 in advanced stage (3B), with a performance status (PS) between 0 and 2.

The maximum follow-up time of the patients was 56.0 months.

The median OS and PFS values were extrapolated for both totality of patients and first- and second-line patients.

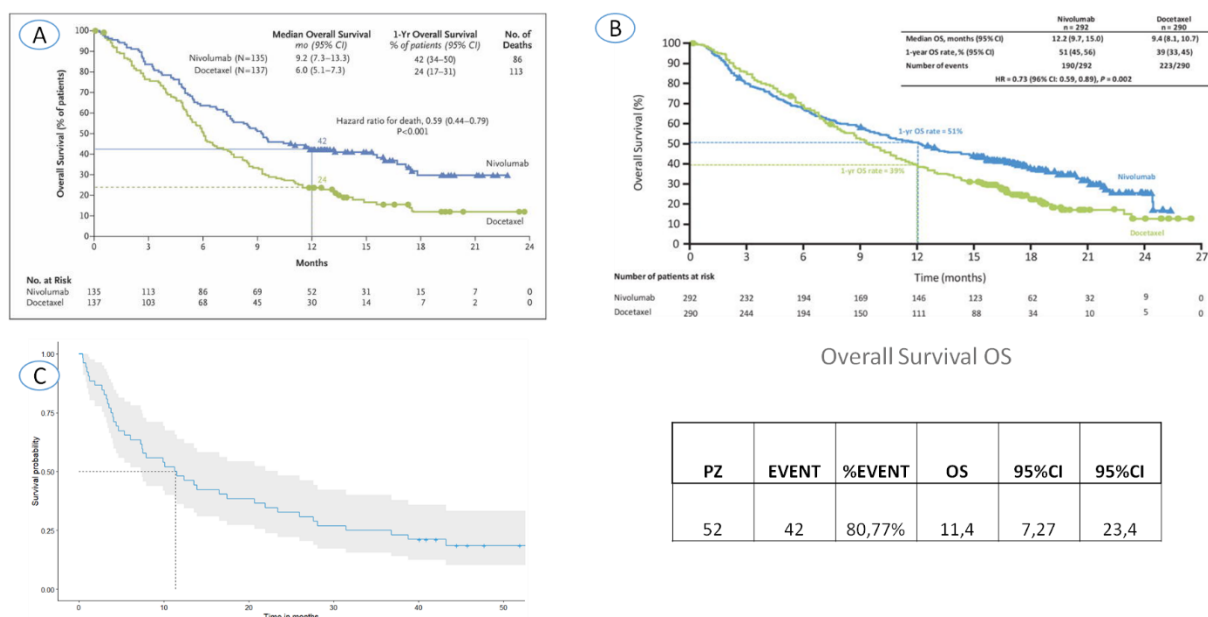


Figure 3.3.1 Kaplan-Meier estimates of overall survival: (A) population of CHECKMATE 017; (B) population of CHECKMATE 057; (C) population of ULSS 3 real world study treated with nivolumab.

Figure 3.3.1 shows the overall survival curve of the 52 patients of ULSS 3 compared with those obtained from pivotal studies CheckMate017⁶⁵ and CheckMate057⁶⁶. These studies verify the performance of nivolumab as monotherapy in patients with squamous or non-squamous histology progressed during or after platinum-based. As can be seen in Figure 3.3.1, the survival curves of nivolumab in the first few months of treatment predict a faster decrease than that of chemotherapy, but there is no long-term stabilization like that of pembrolizumab (Figure.3.1.2).

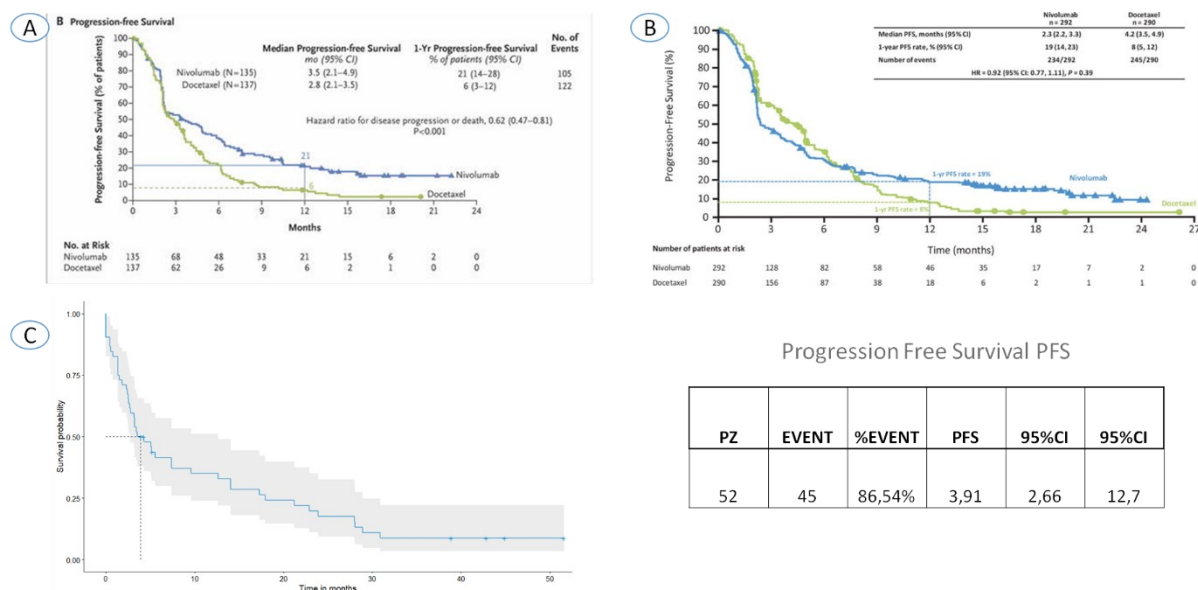


Figure 3.3.2 Kaplan-Meier estimates of progression free survival: (A) population of CHECKMATE 017; (B) population of CHECKMATE 057; (C) population of ULSS 3 real world study treated with nivolumab.

All PFS curves, on the other hand, have a steeper slope than the overall survival curves as can be seen in figure 3.3.2.

On the total population treated with nivolumab different survival curves were calculated by stratifying according to histology, PS ECOG, according to PD-L1 expression to test whether the treatment could give better or worse outcomes relative to these variables. The curves generated resulted in no statistically significant difference.

	REAL LIFE ULSS 3	CHECK MATE 017 (squamous)	CHECK MATE 057 (non squamous)
PATIENTS	52	135	292
MEDIAN AGE	69,9	62	61
RANGE	55-83	39-85	37-84
MALE	34 (65%)	111 (82%)	151 (52%)
WOMEN	18 (35%)	24 (18%)	141 (48%)
3 mg/Kg 14GG	13 (25%)	135 (100%)	292 (100%)
240 mg 14GG	38 (73%)	0	0
480 mg 28GG	1 (2%)	0	0
ECOG 0	12 (23%)	27 (20%)	84 (29%)
ECOG 1	40 (77%)	106 (79%)	208 (71%)
ECOG not reported	/	2 (1%)	0
SQUAMOUS	16 (30%)	135 (100%)	0
NON SQUAMOUS	32 (62%)	0	292 (100%)
NOS	4 (8%)	0	0
PD-L1 TPS not found	32 (61%)	18 (13%)	61 (21%)
PD-L1 TPS ≤1%	11 (21%)	54 (40%)	108 (37%)
PD-L1 TPS 1-5%	2 (4%)	21 (15%)	28 (9%)
PD-L1 TPS 5-10%	3 (6%)	6 (5%)	9 (3%)
PD-L1 TPS ≥10%	4 (8%)	36 (27%)	86 (30%)
ALK POS	1 (2%)	NA	13 (4%)
ALK NEG	42 (81%)	NA	113 (39%)
ALK UNKNOWN	9 (17%)	NA	166 (57%)
EGFR POS	0	NA	44 (15%)
EGFR NEG	34 (65%)	NA	168 (58%)
EGFR UNKNOWN	9 (17%)	NA	80 (27%)
DEATHS	42 (81%)	86 (64%)	190 (65%)
ALIVE	10 (19%)	49 (36%)	102 (35%)
PROGRESSIVE DISEASE	45 (86%)	105 (78%)	234 (80%)
TREATMENT ONGOING	6 (14%)	30 (22%)	58 (20%)
OS	11,4 (7,27 - 23,4)	9,2 (7,3 - 12,6)	12,2 (9,7 - 15,1)
6mSurvival Rates	65,4% (53,1 - 79,7)	/	/
PFS	3,91 (12,7 - 22,66)	3,5 (2,1 - 4,9)	2,3 (2,2 - 3,3)
6mProgressionRates	41,56% (29,97 - 57,6)	/	/
ORR	4 (7,69%)	27 (20%)	56 (19%)
DOR	10,36 (1 - 51,55)	NR (2,9-20,5)	17,2 (1,8 - 22,6)
Duration of observation	18,71 (1- 53,49)	/	/
2° treatment	13 (25%)	/	/
n° administration	21,77 (1 - 69)	8 (1-48)	6 (1-52)

Table 3.3.1 Summary of population characteristics and outcomes of the nivolumab study conducted in ULSS 3 and studies compared.

Below, the most significant differences between the study populations will be reported and the clinical outcomes of ULSS 3 patients treated with nivolumab will be compared with the two registration studies CheckMate017⁶⁵ and CheckMate057⁶⁶. All key data are summarized in the Table 3.3.1.

The number of patients enrolled in pivotal studies CheckMate017 and CheckMate057 is significantly higher than in ULSS 3. The 52 patients selected in ULSS 3 had a higher average age than in the CHECKMATE studies. As with pembrolizumab, the prevalence of patients is

male. In all three studies most patients had a PS ECOG =1, while the remaining part had an ECOG =0.

In the ULSS 3 study both patients with adenocarcinoma and patients with squamous or NOS carcinoma were considered, unlike the CHECKMATE studies where patients were separated according to histology. In ULSS 3 most patients (62%) were diagnosed with adenocarcinoma, 30% with squamous histology, the remainder NOS (not otherwise specified).

Only one patient was found in ULSS 3 with positive ALK rearrangements, while no patients with EGFR-positive mutations were found. In the CHECKMATE 017 study, driver mutations were not investigated, unlike in the CHECKMATE 057 study where 13 and 44 patients were found positive for EGFR mutations and ALK rearrangements, respectively.

In ULSS 3, according to data from the AIFA registers, the majority of patients (61%) had no PD-L1 evaluation for nivolumab. The remaining patients had a PD-L1 expression level $\leq 1\%$, between 1-5%, between 5-10% and only 4 patients (8%) had a PD-L1 $\geq 10\%$. Although PD-L1 expression levels are not required for therapy eligibility in the CHECKMATE studies only 13% and 21% had a PD-L1 that could not be assessed.

Although the percentages of patients who died and patients with progressing disease were higher in ULSS 3 than in the pivotal studies, the median overall survival (OS) values were similar.

The ULSS 3 median overall survival (OS) was 11,4 months (95% confidence interval [CI], 7,27 – 23,4). The ULSS 3 median progression free survival (PFS) was 3.91 months (95% confidence interval [CI], 22,66 - 12,7). Median overall survival (OS) is very similar to those of regulatory studies.

In the ULSS 3 Real life study patients were followed for a maximum period of 53.49 months (almost 4.5 years). Compared to ULSS 3 for the pivotal studies, the observation period was much shorter (almost 24 and 27 months respectively).

Although in the registration studies Objective response rate (ORR) was calculated as the proportion of patients with a complete response or partial response to treatment according to Response Evaluation Criteria in Solid Tumors (RECIST), while in the ULSS 3 study ORR was calculated as the percentage of patients who had no progression, no date of death, and who reported "clinical decision" or "partial response" in the AIFA end-of-treatment motivations, the ORR found in ULSS 3 (7.69%) was much lower than in the CHECKMATE studies (20% and 19%).

3.4 Atezolizumab outcomes

By cross-matching data from the B-MIND database and AIFA registries, 42 patients who received at least one dose of atezolizumab from 2019 to 2020 were selected. All 42 patients received atezolizumab 1200 mg administered intravenously every three weeks.

98% of patients received second-line treatment, while 1 patient received first-line treatment.

Indications monitored by AIFA, involve the use of atezolizumab as monotherapy in pretreated patients with advanced or metastatic NSCLC, in fact all patients in the study with pembrolizumab had a diagnosis of NSCLC: 39 in metastatic stage (IV), 3 in advanced stage (3B), with a performance status (PS) between 0 and 2.

The maximum follow-up time of the patients was 39.78 months.

The median OS and PFS values were extrapolated for both totality of patients and first- and second-line patients.

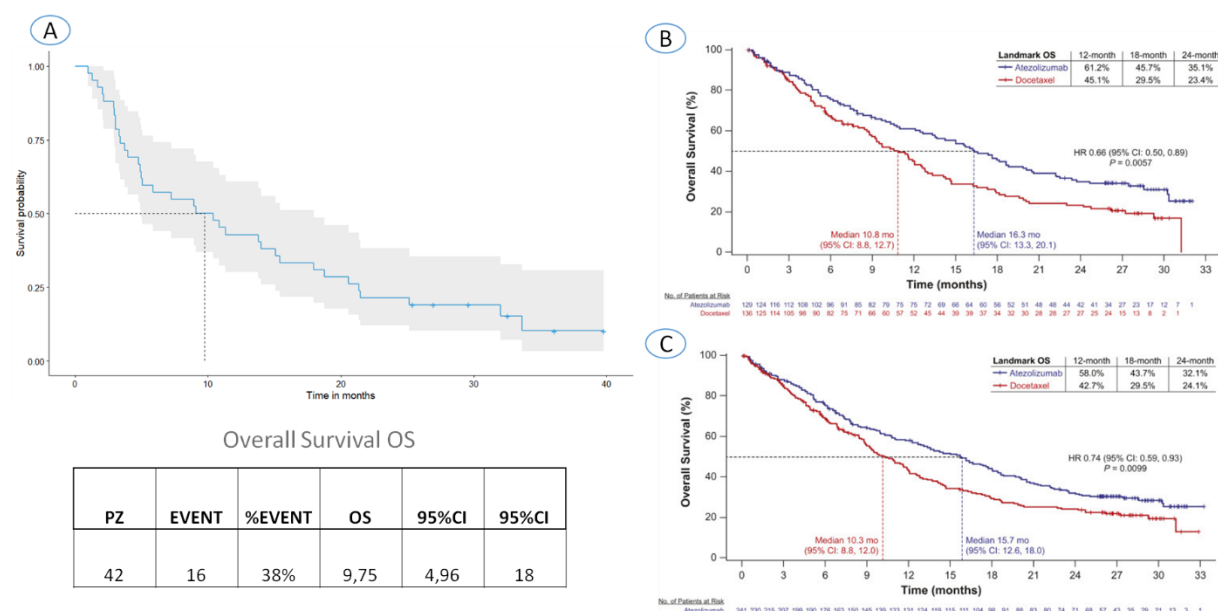


Figure 3.4.1 Kaplan-Meier estimates of overall survival: (A) population of ULSS 3 real world study treated with atezolizumab; (B) population of OAKITT850 with PD-L1 expression on at least 1% of TCs or ICs; (C) population of OAKITT850 with PD-L1 expression on less than 1% of TCs or ICs.

Figure 3.4.1 shows the overall survival curve of the 42 patients of ULSS 3 compared with those obtained from pivotal study OAKITT850⁷¹. The efficacy of atezolizumab, in lines after the first one, was evaluated in this study, in which adult patients with squamous (about 26%) or non-squamous (about 74%) stage IIIB/IV NSCLC with ECOG 0-1 performance status were enrolled. Subjects were included independently from the expression grade of PD-L1. Enrolled

patients received at least two previous lines of chemotherapy. Initially, 850 patients were enrolled (primary efficacy population ITT850). Subsequently, the total number of patients increased to 1225 (secondary efficacy population ITT1225⁷¹) in order to allow a comparison analysis in patients with high PD-L1 expression levels.

As can be seen in Figure 3.4.1, the survival curves of atezolizumab in the first few months of treatment predict a faster decrease than that of chemotherapy, but there is no long-term 74stabilization like that of pembrolizumab (Figure.3.1.2). The survival curves of ULSS 3 show a more sloping trend than those of the OAK study, which instead tend to stabilize over the long term.

On the total population treated with atezolizumab different survival curves were calculated by stratifying according to histology, PS ECOG, according to PD-L1 expression to test whether the treatment could give better or worse outcomes relative to these variables. The curves generated resulted in no statistically significant difference.

	REAL LIFE ULSS 3	OAK ITT850	OAK ITT1225
PATIENTS	42	425	613
MEDIAN AGE	70,43	63	63
RANGE	47-82	33-82	25-84
MALE	33 (78,57%)	261 (61%)	379 (61,8%)
WOMEN	9 (21,42%)	164 (39%)	234 (38,2%)
ECOG 0	13 (31%)	155 (36%)	221 (36,1%)
ECOG 1	28 (66%)	270 (64%)	392 (63,9%)
ECOG 2	1 (2%)	0	0
SQUAMOUS	10 (24%)	112 (26%)	161 (26,3%)
NON SQUAMOUS	29 (69%)	313 (74%)	452 (73,7%)
ADENOSQUAMOUS	1 (2%)	0	0
NOS	2 (5%)	0	0
PD-L1 TPS not found	16 (38%)	0	0
PD-L1 TPS ≤1%	7 (17%)	180 (42%)	180 (42%)
PD-L1 TPS ≥1% <5%	11 (26%)	0	0
PD-L1 TPS ≥5% <10%	1 (2%)	0	0
PD-L1 TPS ≥5% <50%	6 (14%)	0	0
PD-L1 TPS ≥1%	0	241 (57%)	241 (57%)
PD-L1 TPS ≥ 5%	0	129 (30%)	129 (30%)
PD-L1 TPS ≥50%	1 (2%)	72 (17%)	72 (17%)
ALK POS	1 (2%)	4 (0,7%)	2(<1%)
ALK NEG	29 (69%)	315 (51,4%)	223 (52%)
ALK UNKNOWN	12 (29%)	294 (48,0%)	200 (47%)
EGFR POS	2 (5%)	60 (9,8%)	42 (10%)
EGFR NEG	33 (79%)	455 (74,2%)	318 (75%)
EGFR UNKNOWN	7 (17%)	98 (16,0%)	65 (15%)
3 mg/Kg 14GG	13 (25%)	425 (100%)	613 (100%)
DEATHS	36 (86%)	/	/
ALIVE	6 (14%)	/	/
PROGRESSIVE DISEASE	37 (88%)	/	/
ONGOING RESPONSE	5 (12%)	28 (45,2%)	42 (50%)
OS	9,75 (4,96 - 18)	13,8 (11,8 - 15,7)	13,3 (11,3-14,9)
6m-Survival Rates	57,1% (43,99 - 74,3)	/	/
PFS	3,5 (2,1 - 7,36)	2,8 (2,6-3,0)	2,7 (2,4-2,9)
6m-ProgressionRates	38,1% (25,91 - 56,0)	/	/
ORR	11,90%	14,6% (11,4-18,3)	13,7% (11,1-16,7)
DOR	7,02 (1 - 35,70)	16,3 (10,0-26,3)	23,9 (12,8-NE)
Duration of observation	13,27 (1- 39,78)	28 (26-33)	26 (21-33)
2° treatment	9 (21,42%)	/	/
n° administration	10,36 (1 - 48)	/	/

Table 3.4.1 Summary of population characteristics and outcomes of the atezolizumab study conducted in ULSS 3 and studies compare.

Below, the most significant differences between the study populations will be reported and the clinical outcomes of ULSS 3 patients treated with atezolizumab will be compared with the two registration studies OAKITT850⁷¹ and OAKITT1225⁷³. All key data are summarized in the Table 3.4.1.

The number of patients enrolled in pivotal studies OAKITT850⁷¹ and OAKITT1225⁷³ is significantly higher than in ULSS 3. The 42 patients selected in ULSS 3 had a higher average age than in the CHECKMATE studies. As with pembrolizumab and nivolumab, the prevalence of patients is male. In all three studies most patients had a PS ECOG =1, while the remaining part had an ECOG =0, only one ULSS 3 patient has a PS =2.

In the ULSS 3 study both patients with adenocarcinoma and patients with squamous or NOS carcinoma were considered, most ULSS 3 patients were diagnosed with adenocarcinoma 62%, 30% with squamous histology, the remainder NOS (not otherwise specified). In all three studies, the incidence of non-squamous histology is higher than squamous histology.

Only 1 patient was found in ULSS 3 with positive ALK rearrangements instead 2 patients had EGFR mutations. In the OAK study less than 1% of patients were found positive for ALK rearrangements while less than 10% of patients were found positive for EGFR mutations.

In the AIFA monitoring registers of atezolizumab, expression levels are reported considering both tumor cells and tumor-infiltrating immune cells. The PD-L1 levels reported in the ULSS 3 study are only those referring to tumor cells.

In ULSS 3, the majority of patients (38%) had no PD-L1 evaluation for atezolizumab. The remaining patients had a PD-L1 expression level $\leq 1\%$, between 1-5%, between 5-10%, between 10-50%, and only 1 patient (2%) had a PD-L1 $\geq 50\%$. Although PD-L1 expression levels are not required for therapy eligibility in the OAK studies no patient has a PD-L1 that could not be assessed.

The ULSS 3 median overall survival (OS) was 9,75 months (95% confidence interval [CI], 4,96 – 18). The ULSS 3 median progression free survival (PFS) was 3,5 months (95% confidence interval [CI], 22,66 - 12,7). Median overall survival (OS) is very similar to those of regulatory studies.

The ULSS 3 median overall survival (OS) value was lower than in the pivotal studies. The median progression free survival (PFS) is slightly higher in ULSS 3 patients than in patients in the OAK studies.

In the ULSS 3 real life study patients were followed for a maximum period of 13,27 months (almost 4,5 years). Compared to ULSS 3 for the pivotal studies, the observation period was much shorter (almost 24 and 27 months respectively).

Although in the registration studies Objective response rate (ORR) was calculated as the proportion of patients with a complete response or partial response to treatment according to Response Evaluation Criteria in Solid Tumors (RECIST), while in the ULSS 3 study ORR was calculated as the percentage of patients who had no progression, no date of death, and who reported "clinical decision" or "partial response" in the AIFA end-of-treatment motivations, the ORR found in ULSS 3 (11.90%) was much lower than in the CHECKMATE studies (14.6% and 13.7%).

3.5 Comparison between nivolumab and atezolizumab

Nivolumab and pembrolizumab are indicated second-line treatments for advanced and metastatic stages of NSCLC. Given the similar number of study patients treated with nivolumab and atezolizumab, median overall survival and progression-free survival values were compared.

The nivolumab median overall survival (OS) was 11,4 months (95% confidence interval [CI], 7,27 – 23,4) while the atezolizumab median overall survival (OS) was 9,75 months (95% confidence interval [CI], 4,96 – 18). Instead, the median progression free survival (PFS) of nivolumab and the median progression free survival (PFS) of atezolizumab are 3.91 months (95% confidence interval [CI], 22,66 - 12,7) and 3,5 months (95% confidence interval [CI], 22,66 - 12,7) respectively.

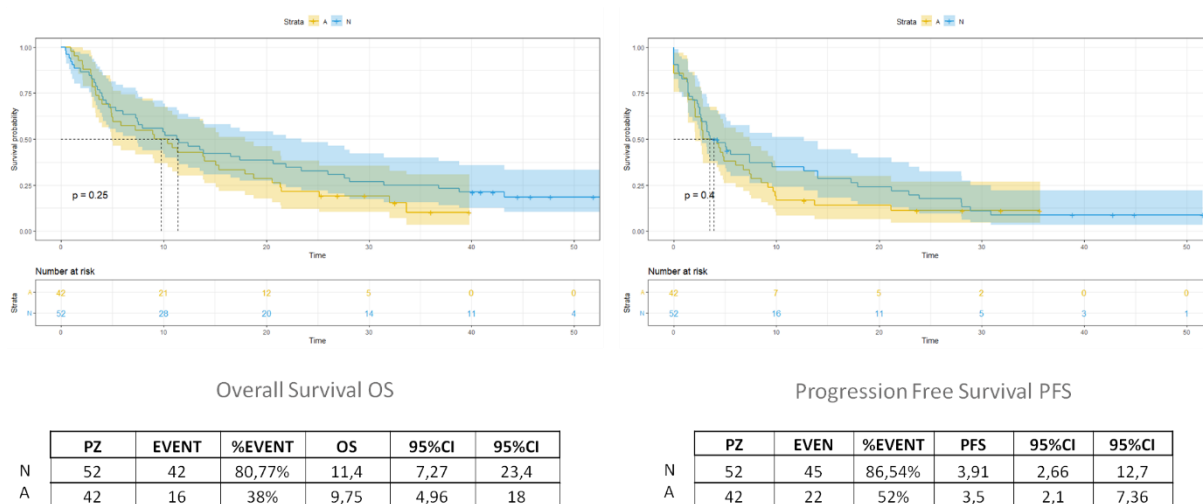


Figure 3.5.1 Comparison of the overall survival and progression free survival curves of atezolizumab and nivolumab.

The comparison of the curves showed no significant difference (p value = 0.4 and 0.5). In the OS estimation graph, it can be seen, as shown in the figure 3.5.1, that the curve of atezolizumab is above that of nivolumab only in the first months of treatment, then around the 5th month of treatment the curves cross and in the long term nivolumab shows a better trend.

3.6 Comparison of clinical outcomes between patients with confirmed and unconfirmed eligibility criteria

The comparison made between the data from the AIFA registers and the data from the pathological anatomy database made it possible to define for each eligibility criterion the frequency of incorrectness.

As explained in Chapter 2.5.2 the comparison allowed to mark the eligibility criteria extracted from AIFA as:

“WRONG”: if the AIFA data had opposite characteristics to those found in the pathology anatomy reports.

“NOT CONFIRMED”: if no information related to that specific characterization was found in the pathology anatomy reports.

“NOT COMPARABLE”: if the patient in the pathology anatomy database of the entire ULSS 3 from 2016 to 2022 had no reports.

“NOT EVALUATED”: patients who in both AIFA and Pathology Anatomy reports present no information regarding Histology, gene mutational status, or PD-L1 expression.

“CORRECT”: patients who present matching information in both AIFA and pathology reports.

Criteria defined as incorrect were those classified as "wrong" and "not confirmed".

		WRONG	UNCONFIRMED	NOT COMPARABLE	NOT EVALUATED	CORRECT	TOT INCORRECT	% INCORRECT ON PT TOT	PROGRESSION DISEASE	% PROG ON INCORRECT	DEATH	% DEATH ON INCORRECT
PEMBROLIZUMAB	Histology	4	3	2	0	74	7	8%	7	100%	6	86%
	EGFR	2	24	2	7	48	26	31%	23	88%	18	69%
	ALK	2	21	2	8	50	23	28%	22	96%	16	70%
	PD-L1	3	19	2	0	59	22	27%	21	95%	17	77%
NIVOLUMAB	Histology	3	7	3	0	39	10	19%	7	70%	7	70%
	EGFR	0	38	3	9	2	38	73%	32	84%	34	89%
	ALK	0	23	3	9	17	23	44%	20	87%	22	91%
	PD-L1	2	5	3	32	10	7	13%	7	100%	6	86%
ATEZOLIZUMAB	Histology	1	2	1	0	38	3	7%	4	100%	4	100%
	EGFR	0	20	1	7	14	20	48%	17	85%	16	80%
	ALK	1	13	1	12	15	14	17%	12	86%	11	79%
	PD-L1	3	3	1	16	19	6	14%	4	10%	4	10%

Table 3.6.1 Frequency of patients with incorrect eligibility criteria about histological, EGFR mutation, ALK rearrangements and PD-L1 evaluation. Criteria defined as incorrect were those classified as "wrong" and "not confirmed". Frequency of patients deceased or with disease progression out of the total number of patients with incorrect eligibility criteria.

As can be seen from Table 3.6.1 for all three antibodies, few patients had wrong eligibility criteria. All the wrong cases related to PD-L1 had different levels than those in AIFA, both higher and lower levels were found than in the pathology reports.

The same for the histology, where distinctive signs of histology, different from those in AIFA, were found.

On the other hand, the EGFR and ALK driver mutations were defined on AIFA as not evaluated, while on the pathological anatomy reports they were identified as negative.

Many more patients have unconfirmed criteria than wrong criteria. In particular, many patients have EGFR and ALK assessments identified as unconfirmed. In this case the information on AIFA was not identified in the reports of pathological anatomy.

Cases of patients with unconfirmed assessments for PD-L1 values are high but only in patients treated with pembrolizumab.

Few cases of patients with not comparable eligibility criteria were found, because only a few patients had no reports made in ULSS 3 from 2016 to 2022.

The cases of patients with eligibility criteria defined on AIFA as not evaluated, for which no information was found in the pathology reports either, are high in patients treated with nivolumab and atezolizumab for PD-L1 evaluation. Not evaluated values were also found for EGFR and ALK mutations.

The frequencies of incorrect values calculated on the total population show that the less concordant values with AIFA are those related to EGFR and ALK mutations, although the incompatibility related to PD-L1 values, particularly in patients treated with pembrolizumab, is of no less importance.

To demonstrate that the choice of therapy based on incorrect criteria led to adverse therapeutic outcomes (progression and death), for each eligibility criteria, the OS and PFS of patients with defined incorrect criteria were compared with those of patients presenting "not comparable," "not evaluable," and "correct" eligibility criteria.

DRUGS	ELIGIBILITY CRITERION	N°CORRECT	N° INCORRECT	p_value_DEATH	p_value_PROGRESSION	OS CORRECT	OS INCORRECT	PFS CORRECT	PFS INCORRECT
PEMBRO	HISTOLOGY	76	7	0.0510	0.0755	15,65	4,18	5,31	1,38
PEMBRO	EGFR	57	26	0.7429	0.1332	9,80	16,75	4,60	8,65
PEMBRO	ALK	60	23	0.8389	0.8279	14,28	11,21	4,52	5,75
PEMBRO	PD-L1	61	22	0.4061	0.8915	15,52	10,16	4,60	5,42
ATEZO	HISTOLOGY	39	3	0.6249	0.7731	10,39	5,85	2,83	4,77
ATEZO	EGFR	22	20	0.8682	0.5977	11,08	8,09	3,50	3,80
ATEZO	ALK	28	14	0.4051	0.3973	13,89	4,93	4,72	2,35
ATEZO	PD-L1	36	6	0.4369	0.2147	9,75	9,91	2,83	7,22
NIVO	HISTOLOGY	42	10	0.2110	0.0674	10,03	26,81	3,50	23,90
NIVO	EGFR	14	38	0.0007	0.0567	39,99	6,66	17,29	2,71
NIVO	ALK	29	23	0.0320	0.1384	13,87	7,96	5,56	2,76
NIVO	PD-L1	45	7	0.6196	0.7334	10,13	31,46	3,55	5,06

Table 3.6.2 OS and PFS median values of patients with defined incorrect criteria were compared with those of patients presenting "not comparable," "not evaluable," and "correct" eligibility criteria.

There were few cases in which the difference between the curves was statistically significant (as can be seen from the p-values in Table 3.6.2), this is due to the different size of the populations compared. Despite the small number of populations with incorrect criteria, it is however interesting to emphasize that, above all, the median values of OS are higher in the populations with correct criteria, while they are lower in the populations with incorrect criteria. With the results obtained, it could not be confirmed that the choice of therapy based on incorrect criteria led to adverse therapeutic outcomes (progression and death).

3.7 Prescriptive appropriateness of pembrolizumab

During the prescribing process, the AIFA platform does not allow the prescription process to be completed unless certain eligibility criteria are filled.

It was essential to verify that the data entered in the AIFA registry agreed with the criteria found on both the B-MIND database and the pathology reports.

The following results concern the analysis of prescriptive appropriateness for pembrolizumab. All patients treated with pembrolizumab had a diagnosis of metastatic NSCLC (TMN staging = IV) with a PFS ≤ 2 . For each patient, the histology of the tumour mass was assessed, although it appeared, from the pathological anatomy data, to be incorrect in 3 patients and not verifiable with the findings in 5 patients.

For all patients, EGFR and ALK mutations were assessed as negative or not performed, although relatively in 26 and 23 patients it was not possible to verify the result of the analysis from the reports.

PD-L1 levels were assessed in all patients although it was not possible to verify the expression level in 21 patients from the pathological anatomy data. While for 3 patients on pembrolizumab therapy, the PD-L1 expression indicated on AIFA did not match in the pathological anatomy reports.

PEMBROLIZUMAB PD-L1	<1%	1 - 49%	$\geq 50\%$	ERR	%
MONOTHERAPY	0	1	52	1	2%
ASSOCIATION	7	16	1	1	4%

Table 3.7.1 Appropriateness of PD-L1 expression levels in first-line patients on pembrolizumab.

In the first-line setting, the criteria required for eligibility for monotherapy refer to a PD-L1 expression $\geq 50\%$. As shown in Table 3.7.1, 1 out of 53 patients treated with monotherapy was found to have PD-L1 expression between 1 - 49%. This case can be defined as a case of prescriptive inappropriateness.

In the first-line setting, the criteria required for eligibility to receive association with pemetrexed or platinum-based chemotherapy refer to a PD-L1 expression $< 50\%$. 1 out of 24 patients treated with monotherapy were found to have PD-L1 expression that did not match the eligibility criteria. As can be seen from Table 3.6.3, 1 patient had PD-L1 $\geq 50\%$. The patient with PD-L1 $\geq 50\%$ would have been eligible for monotherapy.

PEMBROLIZUMAB ASSOCIATION	TOT PT	ERR	%
≠SQUAMOSOUS (pemetrexed)	27	0	0%
SQUAMOSOUS (carbo + paclitaxel)	0	0	0%

Table 3.7.2 Appropriateness of chemotherapy used in association with pembrolizumab.

Another eligibility criterion required in association pembrolizumab treatment is the combination of the type of chemotherapy with the patient's histology. In patients with non-squamous histology the combination with pemetrexed is indicated, in patients with squamous histology carboplatin + paclitaxel is indicated. As can be seen in Table 3.7.2 all patients in combination were treated with the correct antitubercular therapy.

PEMBROLIZUMAB	TOT	Incorrect EGFR %	Incorrect ALK %
1L≠SQUAMOSOUS (ALK e EGFR neg)	65	0 0%	0 0%

Table 3.7.3 Appropriateness of evaluation of EGFR mutations and ALK rearrangements in the first-line patients with non-squamous histology treated by pembrolizumab.

In the first-line treatment of patients with non-squamous histology, the evaluation of EGFR mutations and ALK rearrangements is required to be negative. In first-line patients treated with pembrolizumab, as can be seen in Table 3.7.3, the patients in the study presented a correct evaluation of the driver mutations.

3.8 Prescriptive appropriateness of nivolumab

The following results concern the analysis of prescriptive appropriateness for nivolumab.

All patients treated with nivolumab had a diagnosis of advanced or metastatic NSCLC (TMN staging = IIIB or IV) with a PFS ≤ 2. For each patient, the histology of the tumor mass was assessed, although it was found to be incorrect in 3 patients and unverifiable from the pathological anatomy data in 10 patients.

For almost all patients treated with nivolumab, EGFR and ALK mutations were assessed as negative or not performed, although relatively in 41 and 26 patients it was not possible to verify the result of the analysis from the pathology reports. Only one patient had a positive assessment of ALK rearrangements.

PD-L1 levels were assessed in all patients although the expression level in 8 patients could not be verified from the pathological anatomy data. While for 2 patients receiving nivolumab therapy, the PD-L1 expression indicated on AIFA did not match in the pathological anatomy reports.

3.9 Prescriptive appropriateness of atezolizumab

I seguenti risultati riguardano l'analisi di appropriatezza prescrittiva relativa atezolizumab.

Tutti i pazienti presentavano una diagnosi di NSCLC avanzato o metastatico (TMN staging = IIIB o IV) con un PFS ≤ 2 . Per ogni paziente è stata valutata l'istologia della massa tumorale, nonostante dai dati dell'anatomia patologica sia apparso che fosse errata in 1 paziente e non verificabile con i referti in 3 pazienti.

For all patients treated with atezolizumab, EGFR and ALK mutations were assessed as negative or not performed, although relatively in 20 and 13 patients it was not possible to verify the results of the analysis from molecular diagnostic reports.

PD-L1 levels were assessed in all patients although the expression level in 4 patients could not be verified from pathological anatomy data. While for 1 patient on atezolizumab therapy the PD-L1 expression indicated on AIFA did not match in pathology reports.

ATEZOLIZUMAB		%	NIVOLUMAB		%
1°LINEA	1	2%	7		13%
2°LINEA	41	98%	45		87%

Table 3.9.1 Appropriateness second line treatment with nivolumab and atezolizumab

A required criterion for second-line treatment for both nivolumab and atezolizumab is the presence of previous treatment. As can be seen in Table 3.9.1, 7 patients receiving nivolumab and 1 patient treated with atezolizumab had no previous treatment.

4. DISCUSSION

The cost of cancer care high and rising worldwide, it's a global problem⁷⁹. Also, in ULSS 3 Serenissima, the expenditure for oncological drugs is the most impactful on the total spending of medicines.

The immune check point inhibitors (ICIs) under study have the highest expenditure item after two drugs used in haematology, lenalidomide and daratumumab. The reason for the high cost of these antibodies lies in several factors. These therapies, in the case of Non-Small Cell Lung Cancer (NSCLC), represent the only other therapeutic alternative besides chemotherapy and tyrosine kinase inhibitors, which are only indicated in the presence of targetable driver mutations. They are therefore defined as first-in-class drugs, offering substantial therapeutic benefits through different mechanisms of action than those on the market.

Despite their constant incidence on expenditure, these drugs have fluctuated over the years. In the comparison of spending on immunomodulatory and antineoplastic drug in the years 2021, 2020, and 2019 it can be seen how pembrolizumab shows an increasing expenditure trend, being the only first-line immunotherapy in the years indicated. On the other hand, nivolumab, shows a decrease due to price retraction for the introduction of new therapeutic indications. Atezolizumab, which has a lower price compared to nivolumab, shows an increase compared to 2019, because it is economically preferred to nivolumab given the comparable effectiveness.

As other studies suggest, the prices of anticancer drugs in Italy do not reflect their therapeutic benefit⁸⁰. So further investigations are fundamental to verify whether outcome data obtained after drug marketing would improve the correlation between prices and therapeutic benefit.

Comparing efficacy to drug costs was not the purpose of the study, but the results obtained in the ULSS 3 retrospective studies certainly confirmed that clinical practice often deviates from the efficacy outcomes obtained in pivotal studies. Investigating survival outcomes and comparing them with other studies the effectiveness in ULSS 3 has been verified.

The survival curves obtained from all three studies allowed to extrapolate median Overall Survival (OS) and Progression Free Survival (PFS) values for comparison with the other studies.

From the shape of the curves, for all three antibodies, it was possible to recognize the classic trend typical of immunotherapy, which, compared with chemotherapy, shows a loss of patients in the first months of treatment. This initial behaviour of immunotherapy has not yet been explained as there are no significant predictive elements to define its performance. Within all

survival graphs, the one of pembrolizumab's overall survival shows greater linearity in the long run, and thus greater stabilization of the disease.

The steepness of the curve is even more evident in the PFS curves. This is classic of ICIs that in the early periods may show pseudo-progression or lesion formation and then lead to a constant and vigilant activation of the immune system, except for resistance cases.

Considering pembrolizumab, the results obtained were selected to compare only subpopulations treated in the same way as the pivotal studies. So only first-line monotherapy patients were considered. Among ULSS 3 patients, the PFS and OS values were shown worse than in the KEYNOTE024⁶² and KEYNOTE042⁶³ studies. These differences in efficacy may be explained by the selective enrolment criteria applied in the pivotal studies. Randomized studies with ICIs had strict eligibility criteria, such as patients who had an ECOG PS 0-1, adequate organ function, no history of prior malignancy, and no active CNS metastasis. It is difficult to apply these criteria in everyday clinical practice. It can be seen that patients with PS ECOG =2 were excluded from the pivotal studies, which were instead considered in both real-world studies (ULSS 3 and Cavaille et al.⁷⁸). The same applies to the mean age of the patients: it was found to be higher in the ULSS 3 study.

The discrepancy in results may also be related to differences in numerosity between the two studies. Certainly, a larger court of patients provides more reliable results, and extendable to the whole population. Nevertheless, analyzing the real-life patients allows a more truthful view of drug use and performance. The gap between OS values may also be due to the different observation period, which in the ULSS 3 retrospective study is almost twice as long as in the RCT studies.

Although calculated differently, ORR values were still higher in the registrational studies. Unlike the KEYNOTE studies⁶²⁻⁶³, only the patients with partial response or treatment discontinuation established by the oncologist were found in ULSS 3, whereas no patients with complete response were found unlike in the pivotal studies.

Although it is crucial to evaluate drug outcomes in the post-marketing phases through real-world studies, it is essential to point out the differences with prospective studies, which often do not allow equal comparison of results. In addition to those already listed, one of the substantial differences observed from these studies was the use of databases and not medical records reporting more comprehensive data, useful to explain through a more complete view the appropriate treatment choice. On the other hand, computerized data are much more manageable than paper, and after setting up a method of analysis they allow quickly results.

In contrast to the registrational studies, the results obtained by Cavaille et al.⁷⁸ were in line with those of ULSS 3. Despite the shorter observation period of the French study, the cohort and analysis methods overlap with those used in this paper.

Unlike pembrolizumab, the study conducted on nivolumab presented OS values in line with the two randomized trials CheckMate017⁶⁵ and CheckMate057⁶⁶. Were observed differences related to numerosity and observation time, while PS ECOGs of selected patients were very similar to each other. In both the RCTs⁶⁵⁻⁶⁶ and the ULSS 3 study, patients treated with nivolumab had been previously treated with chemotherapy, but not all treatment modalities were comparable: in the CheckMate studies⁶⁵⁻⁶⁶ patients received 3 mg/Kg of nivolumab every two weeks while in the real-life study most patients (73%) took 240 mg of nivolumab every 2 weeks.

Atezolizumab appeared similarly effective compared with OAKITT850⁷¹ and OAKITT1225⁷³. OS values found, did not differ excessively from those in the RCTs, while PFS values were few months higher. As with pembrolizumab and nivolumab, differences in court numbers were observed. When compared with the pivotal studies, observation times were shorter, as the first uses of atezolizumab were only seen in 2019. As with nivolumab, the general conditions of patients, identified through the PS ECOG were similar in all studies.

For all three antibodies, different survival curves were calculated by stratifying according to histology, PS ECOG, PD-L1 expression to test whether the treatment could give better or worse outcomes relative to these variables. The curves generated resulted in no statistically significant difference in any case.

The comparison between nivolumab and atezolizumab was performed to test whether one treatment was more effective than another considering that the study populations were comparable in terms of numbers and eligibility criteria. Although the trends of the survival curves and the values obtained attributed slightly better results to nivolumab, no significant differences between the curves were identified. Knowing that the observation periods differ by 6 months, this result will be useful for expenditure management. It will be recommended to ULSS 3 clinicians, the use of atezolizumab instead of nivolumab, considering similar clinical evidence but different purchase prices.

Real-world studies define the efficacy of drugs in clinical practice compared to registration studies and thus allow to identify the cases of overuse and prescriptive inappropriateness. Overuse is more like to harm than to benefit a patient and it is an issue that has both clinical

and financial implications. Inappropriateness causes unequal distribution of scarce resources and represents wasted resources with negative returns.

An analysis was made to define, for each patient, whether the eligibility criteria (histologic, EGFR mutations, ALK rearrangements, and PD-L1 expression level), binding the prescribing process, agreed with the therapy administered. Such analysis was allowed by comparisons of the data entered in the AIFA registries with data obtained from the anatomic pathology databases and the B-MIND oncology therapy staging management system. There were few cases of patients presenting data that contradicted pathology reports or had no reports from 2016 to 2022. Many more were patients with unconfirmable eligibility criteria because they were not detected in the reports.

The unconfirmed eligibility criteria were mainly related to ALK and EGFR. The high frequency of data not found in AIFA registries can be explained in two ways: passive mobility and difficulty in obtaining tumor tissue for analysis. By definition, cancer patients are defined as itinerants so they perform examinations, or as in this case molecular diagnoses, at other centres. To confirm this, it was noted that in many reports the result of the molecular diagnosis made at the IOV (Istituto Oncologico Veneto) was attached. In addition, in cases where it is difficult to go for a biopsy, because of the placement of the mass or the patient's condition, new methods of molecular analysis have been introduced that include the analysis of such mutations on circulating cells in the blood. However, these analyses are performed in a few specialized centres in the Veneto region. Therefore, it is possible that some patients have had these tests performed elsewhere and do not appear in the ULSS 3 report.

Problems related to the collection of material for analysis are common in clinical practice. In these situations, priority is given to immunohistochemical analysis for the distinction between malignant and benign tumor masses and for the identification of histology. Only secondarily molecular analyses were performed.

Matching the eligibility criteria for each patient with these two databases, survival values (OS and PFS) were compared between patients with defined correct and comparable eligibility criteria versus patients with discordant or unconfirmable ones. For no eligibility criteria, the analysis produced no significant results between the two populations. Although it has not been statistically proven that the choice of therapy based on incorrect criteria led to adverse therapeutic outcomes (progression and death), it remains of paramount importance that the choice of therapy is made according to the eligibility criteria indicated by guidelines or in monitoring registries.

Although there is no direct clinical effect, inappropriate prescriptive choices, in addition to creating avoidable costs, could go to the exclusion of better therapeutic alternatives.

Therefore, based on the therapy administered, the presence of the eligibility criteria was assessed for each patient during the prescribing activity.

For all patients, the diagnosis of NSCLC could be confirmed. the disease staging, defined by TMN classification, and the patients' general condition, expressed by PS ECOG, agreed with those required by the indications for use.

Regarding pembrolizumab, only 2% of the patients on monotherapy and 4% of patients on combination therapy were inappropriate because they had different PD-L1 expression levels than those required for monotherapy (PD-L1 $\geq 50\%$) or combination (PD-L1 $< 50\%$). All patients treated with pembrolizumab in combination received the correct chemotherapy according to histology type. All patients with first-line non-squamous histology had correctly negative evaluations of ALK and EGFR mutations.

In pembrolizumab therapy, molecular diagnosis is mandatory to allow first-line prescription. 23 and 26 patients presenting a negative evaluation of ALK and EGFR driver mutations on the AIFA registries, respectively, didn't have these data in the pathology reports, and thus the uncertainty remains as to whether they were actually evaluated.

For nivolumab and atezolizumab, being second-line therapies, evaluation of mutational status is not required, but it would still be indicated to allow the patient to access the best present therapy (ex. tyrosine kinase inhibitors). Thus, it is interesting to note that ALK assessment was not confirmed in the pathology anatomy reports for 26 patients treated with nivolumab and 13 treated with atezolizumab. While EGFR assessment was not confirmed for 41 and 20 patients treated with nivolumab and atezolizumab, respectively.

The same applies to PD-L1. From the AIFA registries it seems evaluated in every patient treated with pembrolizumab, while pathological anatomy shows that 3 patients have different expression values and for 22 the expression level cannot be confirmed. For pembrolizumab, it is a major concern that PD-L1 expression levels are not evaluated or found to be inconsistent with laboratory analysis, because it is critical information for access to treatment and for the choice of use as monotherapy or in combination with chemotherapy.

Although PD-L1 levels are not required for treatment eligibility in atezolizumab and nivolumab treatments, given the mechanism of action it would be appropriate to be able to confirm the assessment of PD-L1. However, in 16 and 32 patients, respectively, it was not found.

Other cases of inappropriateness concern the identification of patients treated in first-line treatment with atezolizumab and nivolumab, which, according to indications, are recommended in second-line treatment. In this case, for some of these patients, having started treatment in 2018, it was not possible to identify the first lines of treatment due to a lack of computerization of the oncology therapies management, particularly for the Dolo and Mirano district. Therefore, they cannot be called inappropriate with certainty.

In conclusion, beyond a few patients with values at odds with those required for access to treatment, there is a fair amount of prescriptive appropriateness for the drugs studied.

5. CONCLUSION

In this retrospective study, it was possible to confirm that the antibodies pembrolizumab, nivolumab, and atezolizumab are an effective treatment option for patients with NSCLC in advanced or metastatic stages.

The results of the present study suggest that first line monotherapy of pembrolizumab seems less effective in the real-life population than in the pivotal clinical trials in patients with NSCLC. Median PFS and OS values were found to be lower than expected.

For atezolizumab and nivolumab OS and PFS survival values were in accordance with those of the RCTs presenting the expected efficacy of the premarketing phases.

From the survival curves stratified according to PS ECOG, histologic type, and PD-L1, it could not be confirmed that these are predictive factors, and that the efficacy of these drugs varies according to different values of these factors.

Equal efficacy between atezolizumab and nivolumab was identified, as no significant difference was detected to both OS and PFS. Furthermore, from the similar clinical results, it was determined that, according to the purchase price, atezolizumab would be preferred over nivolumab.

Although it has not been statistically proven that the choice of therapy based on incorrect eligibility criteria led to adverse therapeutic outcomes (progression and death), it remains of paramount importance to choose the therapy according to the eligibility criteria indicated in guidelines or monitoring registries.

Beyond a few patients with values at odds with those required for access to treatment, there is a good level of prescriptive appropriateness for the drugs investigated and few cases of overuse. It can be defined that most of the resources are allocated in a correct way. Considering the expensiveness of such therapies, even the small amount of inappropriately prescribed population, could pose a significant burden on the economic management of pharmaceutical spending. Thanks to this study, it is possible to highlight the areas of inappropriate spending with a consequent improvement the hospital treatment economy. Furthermore, this analysis method could be utilized to improve the economy management for other classes of drugs, other therapeutics areas, and, in the end, different hospitals.

6. REFERENCES

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