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BRONCHIAL PULMONARY CARCINOMA: MOLECULAR AND CLINICAL CHARACTERIZATION – HISTOLOGY, MUTATIONS, STAGING, THERAPEUTICS AND SURVIVAL

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CONTENTS

ABBREVIATIONS

ALK – Anaplastic lymphoma kinase

BAC – Broncho alveolar carcinoma

CNS – Central nervous system

COPD – Chronic obstructive pulmonary disease

CT – Chemotherapy

EGFR – Epidermal growth factor receptor

FDA – United States Food and Drug Administration

GTPase – Guanosine triphosphate hydroxylase

KRAS – V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog

MDT – Multidrug therapy

NSCLC – Non-small cell lung carcinoma

PS – Performance status

RT – Radiotherapy

TKI–Tyrosine kinase inhibitor

WHO– World Health Organization

WT – Wild type

Bronchial-Pulmonary Carcinoma: Molecular and Clinical Characterization - Histology, Mutations, Staging, Therapeutics and Survival

ABSTRACT

OBJECTIVE: Clinical characterization and therapeutic follow-up after histological typing and molecular pathology of Bronchial-Pulmonary Carcinoma

MATERIAL AND METHODS: This retrospective study is supported by information collected from a 2011-2013 data basis provided by *Instituto de Anatomia Patológica e Patologia Molecular of Faculty of Medicine of Coimbra,* concerning histological typing and EGFR, KRAS and ALK mutation status in biopsies, and subsequent follow up of patients, treated at University *Hospital of Coimbra, Instituto Português de Oncologia of Coimbra and Hospital of Guarda.*

RESULTS: Data corresponds to 56 patients with bronchial-pulmonary carcinoma, most (64.3%) of whom were male. Adenocarcinoma was the most common histological type (66.1%), followed by pleomorphic (8.9%), epidermoid (7.1%), adenosquamous (7.1%), large cell (5.4%), sarcomatoid (3.6%) and mucoepidermoid (1.8%) carcinomas. In men, the most common histological type was adenocarcinoma (66.7%), as well as in women (65%). The mean age at diagnosis was 66 years old. About 62.5% had prior history of smoking. 64.3% presented stage IV at diagnosis, 14.3 % IIIB, 7.1 % IIIA and the remaining 14.3% was classified as stage I or II. In 29 cases patients showed mutated epidermal growth factor receptor, comparing with 27 biopsies wild type. About 39.3% received tyrosine kinase inhibitors and 32.1% were treated with chemotherapy combined with radiotherapy.

CONCLUSIONS: The study showed higher incidence of bronchial pulmonary carcinoma in men. Adenocarcinoma was the most frequent histological type either in men and women. Smoking habit was prevalent. The majority of patients with mutated status for epidermal growth factor receptor received tyrosine kinase inhibitors (19). For patients wild type, conventional chemotherapy was applied in most cases (19) .The overall survival for patients carrying mutated epidermal growth factor receptor is higher, comparing with the ones wild type, and this result has statistical significance. Radiotherapy was used in association with chemotherapy or alone in palliative therapeutic measures. Most patients presented advanced stages at diagnosis and so curative options were applied only in few cases (11).

Keywords: EGFR, ALK, KRAS, TKI treatment, Adenocarcinoma, Survival

Introduction

According to the World Health Organization (WHO) and based on World Cancer Report 2014, lung cancer is currently the malignant tumor with the highest mortality (1.59 million deaths) followed by liver (745 000 deaths), stomach (723 000 deaths), colorectal (694 000 deaths), breast (521 000 deaths) and esophageal (400 000 deaths) cancer. The 5 most common cancers diagnosed in 2012 among men were lung, prostate, colorectal, stomach, and liver cancer. Among women, breast, colorectal, lung, cervix, and stomach cancer had this decreasing incidence.[1]

Smoking habit continues to be the most important risk factor for lung cancer causing around 70% of global lung cancer deaths.[1]

In clinical practice, bronchial-pulmonary carcinomas are classified as small cell carcinoma, squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. According with 1999/2004 classification, other less frequent histological types are adenosquamous carcinoma, pleomorphic carcinoma, sarcomatoid carcinoma and carcinoid tumor.[2]The actual classification for lung cancer, beyond establishing criteria for recognition of patterns and histological types, has also commitment with prognosis and predictive studies of molecular pathology that showed a correlation between morphology and prognosis outcome directing personalized therapy.[3] Thus, tumors undergo epidermal growth factor receptor (EGFR) gene analysis.

EGFR is a transmembrane protein with cytoplasmic kinase activity that transduces important growth factor signaling from the extracellular milieu to the cell. More than 60% of epidermoid carcinoma and adenocarcinoma express EGFR and therefore, it became an important therapeutic target for the treatment of these tumors. Tyrosine-kinase inhibitors (TKIs) are particularly effective in patients whose tumors harbor activating mutations in the tyrosine kinase domain of the EGFR gene.[4] So, in 2003 and 2004, gefitinib and erlotinib, respectively, were approved by the United States Food and Drug Administration (FDA) for advanced adenocarcinomas that had not responded to previous conventional chemotherapy (CT). [5] Recent trials have suggested that for advanced adenocarcinoma patients with EGFR mutant status, initial therapy with TKIs instead of conventional CT may be the best choice of treatment.[4] Thus, most guidelines have been updated with the consensus that an EGFR mutation is the strongest predictive factor for TKI treatment .[6] Therefore, mutation testing is mandatory to identify these patients, given that selection based only on clinical-pathologic characteristics is incomplete.[4]

Bronchial – Pulmonary adenocarcinomas also harbor activating mutations in the downstream GTPase, KRAS, and mutations in EGFR and KRAS are often mutually exclusive. KRAS mutations occur more frequently in lung adenocarcinomas (30%) and less frequently in the squamous cell carcinoma subtype (5%). Unlike EGFR mutations, which occur more frequently in tumors of never-smokers, the presence of a KRAS mutation cannot be predicted on the basis of smoking history alone.[7] Recent studies indicate that patients with mutant KRAS tumors fail to benefit from adjuvant CT and do not respond to EGFR inhibitors.[8] In the vast majority, KRAS mutations are found in tumors EGFR wild type (WT) and without ALK alterations. Therefore, KRAS mutation defines a distinct molecular subset of the disease.[9]

Some tumors contain an inversion in chromosome 2 that juxtaposes the 5' end of the EML4 gene with the 3' end of the ALK gene, resulting in the novel fusion oncogene EML4-ALK. ALK fusion oncogene defines a molecular subset of tumors with distinct clinical and pathologic features. Patients presenting ALK rearrangement are relatively young, never or light smokers and diagnosed with adenocarcinoma. For those with advanced or metastatic tumors with a characteristic ALK fusion oncogene it is recommended initial treatment with the ALK inhibitor crizotinib rather than CT.[10]

When using CT only, advanced-stage (stage IIIB or IV) tumors are treated with a double combination of a platinum compound (cisplatin or carboplatin) with gemcitabine, vinorelbine, or a taxane (paclitaxel or docetaxel) according with reference regimens.[11]

Material and Methods

For this retrospective study, biopsies were randomly selected and then a follow up was performed in order to characterize the sample. Thus, patients' files were accessed and variables were selected. Gender, age, smoking habit, histological type, mutations, staging, treatment, and survival were analyzed. For the description of the categorical variables (gender, smoking habit, histological type, mutations, staging and treatment), distributions of frequencies was used. For the continuous or numerical variables (age), measurements of central tendency were performed. Overall survival was obtained using the Kaplan-Meier method. Survival stratified by mutation status, smoking habit and staging was also obtained. The differences among the survival curves were determined using the log-rank test, and the level of significance was set at 5%. The statistical analyses were performed using the MedCalc® program for Microsoft Partner, Application Development.

Table 1 – Distribution of gender, smoking habit, gene mutations and staging according to histological type

HISTOLOGICAL TYPE	RF (%)	Male/Female	Present or Past smokers – male/female	EGFR	KRAS	ALK fusion	STAGES						
							IA	IB	IIA	IIB	IIIA	IIIB	IV
Adenocarcinoma	37 (66,1%)	24/13	18/4	18	6	1	4	2	-	-	3	5	23
Pleomorphic carcinoma	5 (8,9%)	2/3	2/1	3	1	1	-	1	-	-	1	-	3
Epidermoid carcinoma	4 (7,1%)	4/0	4/0	2	-	-	-	-	-	-	-	1	3
Adenosquamous carcinoma	4 (7,1%)	2/2	2/0	1	1	-	-	-	1	-	-	2	1
Large cell carcinoma	3 (5,4%)	2/1	2/0	3	-	-	-	-	-	-	-	-	3
Sarcomatoid carcinoma	2 (3,6%)	2/0	2/0	1	-	-	-	-	-	-	-	-	2
Mucoepidermoid carcinoma	1 (1,8%)	0/1	0/0	1	-	-	-	-	-	-	-	-	1

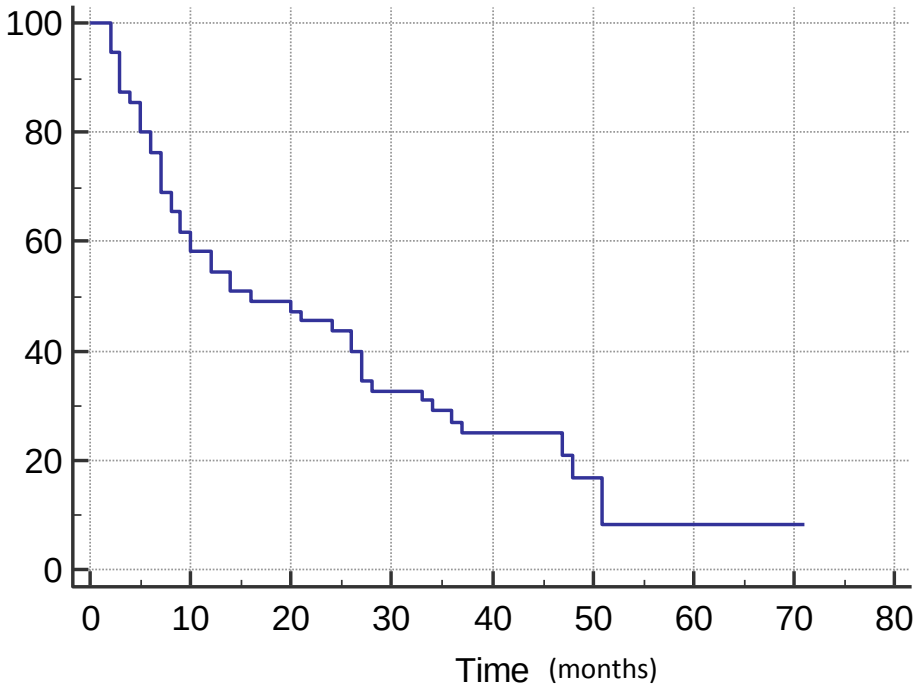

Fig1. Kaplan Meier overall survival curve for the entire population (mean survival time: 24.55 months, CI 95% 18.70 – 30.40)

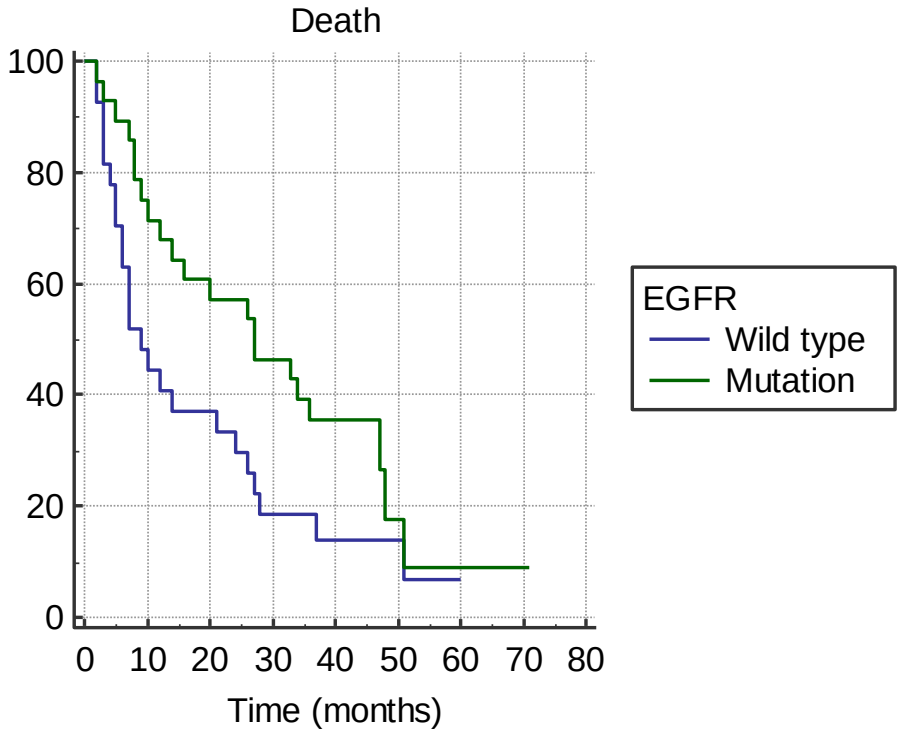

Fig2. Kaplan Meier survival curves comparing patients with EGFR mutation and EGFR wild type (P = 0.0486; CI 95%)

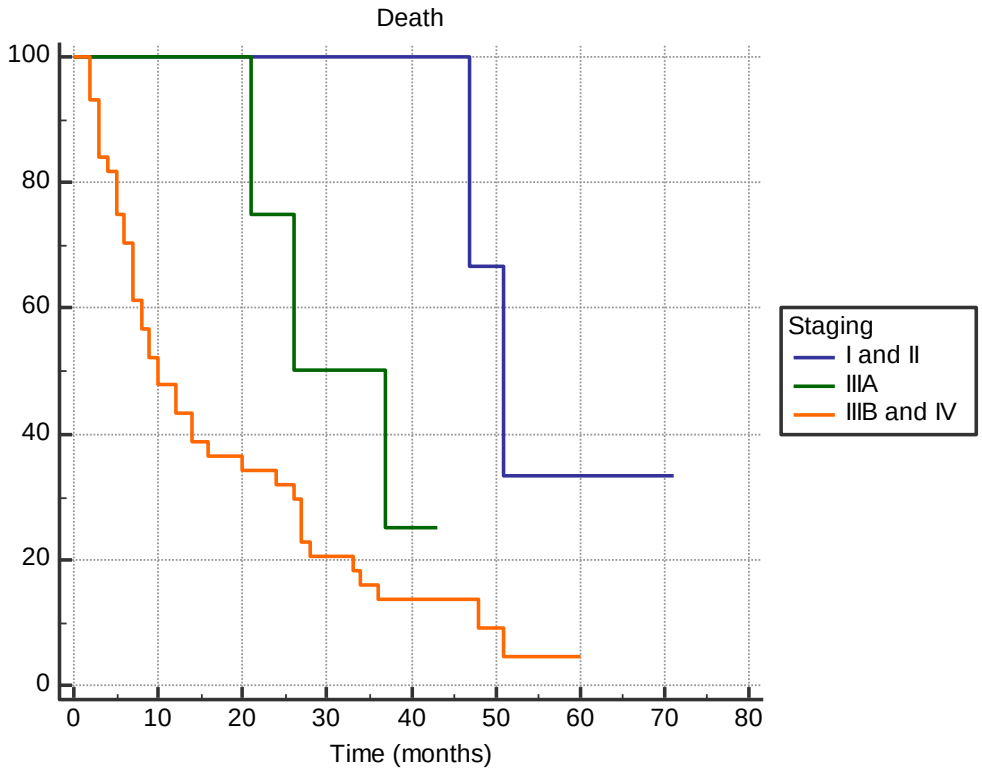

Fig3. Kaplan Meier survival curves comparing staging groups (mean survival time: I and II – 52.67 months, IIIA – 32.00 moths, IIIB and IV – 16.80 months; P = 0, 0018; CI 95%)

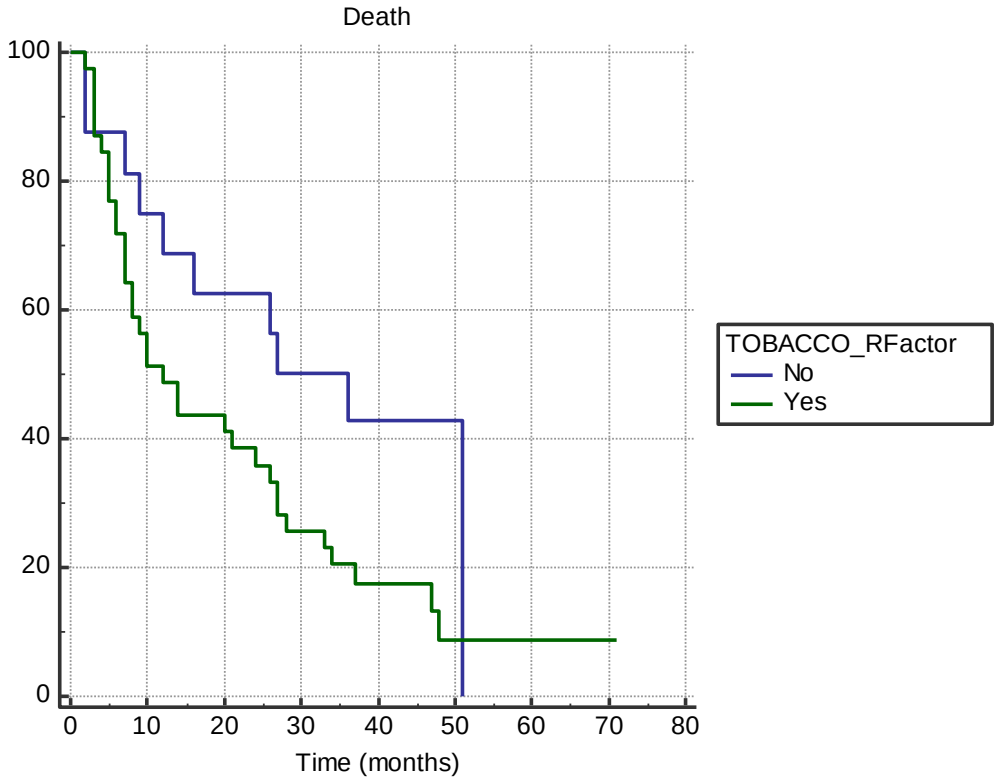

Fig4. Kaplan Meier survival curves comparing 2 groups: tobacco intake and/or other risk factor for lung carcinoma versus non-smokers and no risk factor described (P = 0, 0869; CI 95%)

Results

Data corresponds to 56 patients with lung cancer, most (64.3%) of whom were male. The most common histological type was adenocarcinoma (66.1%), followed by pleomorphic carcinoma (8.9%), epidermoid carcinoma (7.1%), adenosquamous carcinoma (7.1%), large cell carcinoma (5.4%), sarcomatoid carcinoma (3.6%) and mucoepidermoid carcinoma (1.8%). (Table 1) In men, the most common histological type was adenocarcinoma (66.7%), as well as in women (65%). The mean age at the moment of diagnosis was 66 years old. About 62.5 % had prior history of smoking, especially men (83.3%) and the most common histological type in smoking patients was epidermoid carcinoma (100%) and in non-smokers was adenocarcinoma, especially in women (60%). The EGFR mutation status (29) was not associated with smoking; we found 13 non-smokers and 16 smokers, presenting the mutated status. KRAS mutation was tested in 24 adenocarcinomas resulting 6 positive biopsies; the remaining tested KRAS was 4 epidermoid carcinoma with no positive result, 3 pleomorphic carcinoma resulting in 1 positive biopsy and also 3 adenosquamous carcinoma with 1 positive biopsy. ALK fusion oncogene was tested in 11 patients and was positive in 2 cases (1 adenocarcinoma and a pleomorphic carcinoma with a component of adenocarcinoma). These patients were relatively young (28 and 57 years old) and received crizotinib. Concerning staging, 64.3% presented stage IV at the time of diagnosis, 14.3% presented stage IIIB, 7.1% presented stage IIIA, and the remaining 14.3% was classified as stage I or II. (Table 2) In 65.5% of cases, patients who showed EGFR mutation received TKIs. From those with EGFR WT, 70.4% received conventional CT only. About 32.1% were treated with CT combined with radiotherapy (RT). Some (3.7%) were submitted to surgery alone or surgery accompanied by CT, with or without RT (19.6%).The most common patients' complaints along the time where dyspnea (46.4%), cough (39.3%), the pain from metastasized bone cancer (37.5%), chest pain (35.7%) and blood-tinged sputum (30.4%). The overall survival for this population was 42.4 months. (Fig.1)

Discussion and Conclusions

In this study it was obtained a 1.8:1 male/female ratio for bronchial-pulmonary carcinoma. This result is identical to the one registered in the United States, where the lung carcinoma was the first cause of death in men and also became the first cause in women surpassing breast cancer.[12] In men we found a higher index for the use of tobacco, what can clearly justify this ratio. Tobacco is the major cause for lung carcinoma in as many as 90% of patients.[13] For never smokers, different etiology and carcinogenic processes are likely to be

involved, but current understanding is still limited.[14] Data in this study corresponded to 62.5 % of present or past smokers. The risk of lung cancer declines slowly after smoking cessation and this long term risk explain the development of almost 50% of lung cancer cases in past smokers.[13] Nevertheless in some, especially women, a great percentage develops carcinoma and do not smoke. Therefore other causes should be described such as passive smoking or the occupational exposure to carcinogenic substances that are related with lung carcinoma, according to literature. Our data indicates that in three non-smoking patient, it was described occupational exposure - one patient worked in a foundry, other in plastic manufacturing and the third as a mechanic with car smoke exposure.

The predisposition to develop lung tumors also seems to increase in some respiratory diseases such as chronic obstructive pulmonary disease, tuberculosis and in all respiratory diseases that curses with fibrosis.[15] In this study 3 smoking patients were diagnosed with chronic obstructive pulmonary disease (COPD) and one of them also had tuberculosis in the young age; it was too found a nonsmoking female, diagnosed with scleroderma and consequently pulmonary fibrosis, that developed lung carcinoma. These risk factors should be described and well clarified to understand the reason behind the development of the disease in each individual. For instance, 22 patients included in this study lived in Guarda, a region where the presence of radon, which is a radioactive gas produced as a result of uranium decay, can be present in soil, air and water and that in high concentrations is related with lung carcinoma; radon exposure is a well-established risk factor for lung cancer in uranium miners. Approximately 2-3% of lung cancers annually are estimated to be caused by radon exposure. Radon is associated with granites and the existence of granitic source rocks, which are rich in uranium crystals and are present in this area. Has no color, taste or smell, does not react chemically and so it is not detectable by the senses and upon release of the soil can accommodate up inside houses. Household exposure to radon, however, hasn't clearly shown to cause lung cancer [13] but various studies show appreciable hazards from residential radon, particularly for smokers and recent ex-smokers being responsible for about 2% of all deaths from cancer in Europe.[16]

Asbestos is clearly defined as another carcinogenic substance that increases the risk of developing lung cancer by as much as 5 times; in this study in one patient, besides the smoking habit it was described the asbestos exposure. This can act as a synergism for the development of the disease and in these persons the smoking habit and the history of asbestos exposure approaches 80-90 times comparing with control populations.[13] Asbestos also are related with mesothelioma and in other case, also a smoking patient developed

adenocarcinoma and synchronically mesothelioma. Evidently for this case the prognosis was not favorable and the patient only had 5 months of living since the diagnosis was made.

Additionally, also the radioisotope exposure, such as radioactive iodine is related with the increase for developing other carcinomas such as lung cancer.[17] In our study, a female patient, non-smoker and with no described occupational exposure, treated with radioactive iodine for thyroid cancer (papillary carcinoma), developed adenocarcinoma 15 years later.

In this research, the mean age of diagnosis was 66 years old. Lung cancer occurs predominantly in persons aged 50-70 years; it is very low until age 39 in both sexes.[13] In this study it was found one smoking female patient diagnosed with adenocarcinoma at 39 years old and one smoking male with 28 years old, also an adenocarcinoma. The probability for developing lung cancer reaches the peak among those older than 70 years. In this research 19 patients were older than 70.

The most common histological type in this study was adenocarcinoma (66.1%), as verified in the rest of Europe and in the United States[12], followed by pleomorphic carcinoma (9.1%), epidermoid carcinoma (7.1%) and adenosquamous carcinoma (7.1%). Others like large cells, sarcomatoid and mucoepidermoid carcinomas were less frequent. Adenocarcinoma arises from the bronchial mucosal glands and is the most frequent non-small cell lung cancer representing 35-40% of all lung cancers. It is also the subtype observed most commonly in persons who do not smoke.[13] Broncho alveolar carcinoma (BAC) is a distinct subtype of adenocarcinoma with a classic manifestation as an interstitial lung disease on chest radiograph. BAC keeps being controversial as considered an in situ carcinoma in WHO classification and then reported with metastases in some series. BAC is multifocal and often bilateral. However in the presence of larger lesions with a mixed non- mucinous and mucinous type, when desmoplastic reaction is present with acinar invasion and then related to lymph node metastases is no more a BAC in WHO classification but a mixed type adenocarcinoma.[3]It is well stated that pulmonary adenocarcinomas in women have a better prognosis.[3]The response to TKI-based therapy and EGFR mutations is more frequently found in nonsmokers, female patients, adenocarcinomas, and lung cancers showing bronchi alveolar features.[7] In this research concerning adenocarcinomas, 13.5% were described as BAC and 16.2% as mixed type adenocarcinoma. In women the adenocarcinoma is much more prevalent then epidermoid carcinoma and in line with this, it was registered in this study all the epidermoid cases in men. Women also had a higher rate of carcinoma in non-smokers (45%) then men (16.7%) and these facts can distinguish a group of population with

adenocarcinoma consisting in women and non-smokers. NSCLC in women is a growing health concern in Western countries. The disease is reported as a peculiar pathological and molecular entity: women are more affected by lung adenocarcinoma and the tumor arises among never or light smokers with higher frequency than in men.[18] Historically, epidermoid carcinoma is related with smoking habit, mostly male gender, and also here in all cases the patients were smokers and men. However, differences in tobacco smoking habits (filters, light tobacco, cigarettes) appear to favor the development of distal bronchiolar and alveolar carcinogenesis at the expense of proximal squamous cell carcinoma [5]what justifies the increasing of adenocarcinoma surpassing epidermoid carcinoma in the last years.

Concerning mutations, EGFR mutation is found in almost half of the biopsies, which is verified also in numerous series [19]; according to these no prior history of smoking is common as well as the female gender. In this study adenocarcinomas with EGFR mutation are about 48.6% but the mutation was also found in the other histological types. Among adenocarcinomas, mutations were mostly associated with nonsmokers (55.6%) rather than smokers. So, decreasing mutation rates with increasing direct tobacco exposure was observed. Therefore, EGFR mutations were prevalent in lung adenocarcinoma suggesting that it plays an increasing oncogenic role with decreasing direct tobacco damage.[14]

For KRAS, mutations are found predominantly in adenocarcinomas. In this study 34 biopsies were performed and 8 of them where positive, 6 in adenocarcinomas and 1 in a pleomorphic carcinoma and the other in adenosquamous carcinoma. According with literature, KRAS is less common then EGFR, being present in 25% of cases and also in this characterization only 23% for tested KRAS was mutated. KRAS when occurs together with EGFR muted, appears to confer some resistance to TKIs. The 37 cases of mutated EGFR and KRAS occurred in mutually exclusive tumors, in the majority of the biopsies; only in 3 cases they occurred together. In these cases, comparing with those with associated WT KRAS and mutated EGFR, we found no difference in overall survival. However, we should not take any conclusions from this, because the 3 positive biopsies for mutated KRAS and EGFR belonged to very distinct patients: different stages at diagnosis (stage IA, IIIB and IV) and one of them had mesothelioma associated with adenocarcinoma and therefore the survival, in this case, was only 5 months.

EGFR mutation is only carried by about 44 to 66% of female and non-smokers. Therefore in 2007 ALK rearrangements were identified and target therapeutic (crizotinib) was approved by FDA in April 2010. Patients with ALK rearrangement tend to be young, with a

younger median age of diagnosis comparing with EGFR mutations and there is a higher proportion of non-smokers or light smokers. The vast majority present with adenocarcinoma and have no sex preference for male or female.[20] However, in some studies, were encountered ALK positive patients older than 60 years. Thus, elderly patients should not generally be excluded from ALK testing. [21] In our study, ALK rearrangement was tested in 11 cases; all relatively young patients. The ALK rearrangement was positive in a 57 years old female patient with a pleomorphic carcinoma with a component of adenocarcinoma, and in a smoking young male (28 years old) with adenocarcinoma.

In the majority of centers, the diagnosis was made in advanced stages (IIIB and IV), and in this study the same was verified with 78.6% of cases; when that occurs symptoms are always present comparing with early stages where the patient is asymptomatic. [13]

According with guidelines for patients with disease stage I or stage II surgery provides the best chance for cure. [15] In this study we verify that patients diagnosed in these stages (14.3%) where submitted to lobectomy and the three-year survival rate in these cases is quite high, 100 %.

For patients with tumors in stage III, where there are different therapeutic options such as surgery, RT or CT it is required a multidisciplinary evaluation. To stage IIIA the therapeutic options should be organized according to the tumor location in view of the surgical resection.[15] In this research for this condition, it was found 1 patient that was submitted to surgery and adjuvant CT. In this case, for EGRF status we find WT and so no TKIs were administered. The survival was about 26 months.

For patients in unresectable IIIA or IIIB stages without pleural or pericardial spilling, the choice of chemo-radiotherapy is superior to RT or CT isolated.[15] According to our data, in unresectable IIIA or IIIB stages, 44.4% of patients received CT plus RT concomitantly. Patients in stage IIIB, the tumor with T4, N0-1 (T4 tumors satellite lesions) corresponds to a group potentially curable with surgery. Resection is recommended, followed by CT. In this study only one patient in stage IIIB was submitted to surgery + CT and the survival was about 14 months.

For CT in NSCLC for advanced disease (patients in stage IIIB (N3 supraclavicular) or stage IV (M1a with pleural or pericardial), met analyzes have shown that when compared with best supportive therapy, CT resulted in increased survival of patients. For NSCLC in stage IV the goal of the therapy is to increase survival, control symptoms and to improve

quality of life. Therefore, 1st line CT recommended for patients with good performer status (PS) is a combination of platinum derivatives with other drugs of 3rd generation such as gemcitabine, vinorelbine, paclitaxel or docetaxel. For patients with non-squamous histology, in combination with pemetrexed, cisplatin demonstrated significant benefit in overall survival for patients with good condition. In our study and for advanced stages, 25 patients received a combination of platinum plus pemetrexed, what corresponds to 67.6 % of advanced stages. For patients with EGFR mutation it is also used the TKIs (gefitinib) in 1st line. [15] In our research about 7.1% received TKIs as a first line. Monotherapy with a 3rd generation drug, is a reasonable choice, with less iatrogenic effects and is an alternative for patients with PS 2 or elderly (age>70 years) and in Portugal about 35- 40% of patients with lung cancer has more than 70 years old, significant comorbidities and high risk of toxicity for CT associations. So, it is used vinorelbine or gemcitabine. [15] In the study, 3 patients received oral vinorelbine as an alternative; 2 patients with 82 years old and 1 with 76 years old.

The response to the 1st line CT is often short and progression may occur within 4-6 months after finishing the 1st line therapy. Guidelines recommend docetaxel, pemetrexed or erlotinib in monotherapy after failure of a 1st CT regimen. Docetaxel is more effective than best supportive care, vinorelbine and Ifosfamide. Pemetrexed as an identical overall effectiveness compared to docetaxel, with significant lower toxicity and also greater efficacy and survival in non-squamous cell carcinomas. Erlotinib is indicated, for all histological types, after failure of at least one CT regimen. [15] In this study, after a 1st line, 28.9 % received a 2nd line with docetaxel or pemetrexed, about 26.7% received erlotinib.

For 3rd line CT, erlotinib is the only approved agent for this disease without restriction phase. [15] In the research it was observed that 11.1 % received erlotinib as 3rd line.

Despite the aggressive use of CT, the prognosis for patients with NSCLC advanced disease is poor with average survival of 9-12 months in stage IV. [15]

Kaplan Meier curves (Fig.1-Fig.4) showed a low overall survival for this population, mostly in advanced stages as was expected. Comparing mutation status, the overall survival was quite higher in those with muted EGFR comparing with EGFR WT, and these results showed statistical significance. The tobacco exposure, as well as risk factors exposure also showed a tendency to aggravate the overall survival comparing with those who do not smoke.

RT applied in stage IV is performed with palliative intent. In this analysis, patients in stage IV were in the majority of cases (30.6%) treated with RT for brain and for bone

metastases. For brain metastases, when it is single and ressecabale or partially ressecabale it is indicated surgery followed by holocraneane RT. [15] In this research one patient was submitted to metastasis resection resulting paresis. For multiple metastases it is recommended holocranean RT. About 13.9 % was submitted to RT for brain metastasis.

Bone metastases external radiation may be employed with the aim of preventing a pathological fracture, getting pain relief or avoid medullar compression. In these conditions 22.2% were submitted to RT for bone metastases and in the remaining, the option was pharmacological therapeutics such as durogesic® or others related.

Concerning the most common patient's complaints/intercurrences along the time for decrescendo order it was verified dyspnea (46.4%), cough (39.3%), pain from metastasized bone cancer (37.5%), chest pain (35.7%) and blood-tinged sputum (30.4%).

After reuniting and analyzing all data we understand that in this research, we found a large heterogeneity for patients regarding histological, molecular and clinical features. When we identify histology and then mutation status, we can stand in different levels concerning the clinical process. Therefore, the majority of tested samples for the presence of mutation belonged to patients in advanced stages for the disease, were the surgery was not an option anymore and so it was fundamental to apply target methods to treat them. Testing for the presence of mutations, such as EGFR, KRAS or ALK rearrangement is fundamental to direct the treatment. Conventional CT allied to TKIs, when the EGRF is mutated, was the first line treatment for these patients. Those in early stages were much lesser, because as we already referred, the diagnosis is made much more frequently in advanced stages. The early stages are usually asymptomatic and the diagnosis is usually suspected in a routine examine, which means that it is an imagiologic finding. So, these patients were initially treated with surgery but then, in some phase of the disease it was verified a progression or a recidivating and therefore, mutation status was performed in order to offer more options to these patients, such as erlotinib in mutated EGFR or crizotinib for ALK rearrangement. In present time, testing for the mutations is a standard step performed at time of diagnosis to best direct the treatment in any phase of the disease. We understand that for EGFR and KRAS, this process is a routine but since 2009, when crizotinib was authorized by FDA, testing for ALK rearrangement is also important especially in patients with some especial features. The knowledge of the status mutation is fundamental to interview in good time for any stage of the development/progression of the disease.

In future studies it is relevant to continue to search and to test for targeted therapies. Obviously, a standard regimen is adequate but treatment “on series” is not adjusted for all the various features that can be finding among the molecular and clinical characterization of the bronchial pulmonary carcinoma. Further studies targeted on ALK fusion are extremely important because we realize that this affects a young part of population and therefore, offering them more effective therapies is crucial.

At last, also some words for the importance of lung cancer screening. Various strategies for lung cancer screening were studied in order to achieve an earlier diagnosis, but despite some occasional benefits, no demonstrated advantages in terms of lower mortality were showed. With the publication of the results of the National Lung Screening Trial, which was supported by the National Cancer Institute, it was reached a new paradigm in relation to screening of these malignant tumors. The results showed that computerized tomography screening has found a decrease in mortality of 20% for lung cancer, in relation to the radiograph which, statistically, was very significant, and never before achieved, requiring 320 computerized tomography scans to prevent 1 death due to lung cancer. The study, opened a window of opportunity for lung cancer screening at an early stage, but a few problems arise and are concerned with the high number of lesions that need further investigation and then turn out to be benign, the emotional charge that this fact entails, the costs of screening, whether it should be extended to other groups of target populations, the fact that a negative computerized tomography scanning can motivate to continue to smoke, the possible risks in the long term radiation, the need to define how often the imaging studies should be obtained and how to monitor the follow-up of screened. There is a need to consolidate the benefits and characterize the possible risks of this approach, which should be based on centers with high quality imaging and where it is possible to have a multidisciplinary team that integrates imagiologists, pathologists, pulmonologists and thoracic surgeons to reach a consensus on the value of low-dose helical computerized tomography screening for populations at risk. [22]

References

1. Stewart, B.W., et al., *World cancer report 2014*. 2014, Lyon, France, Geneva, Switzerland: International Agency for Research on Cancer, WHO Press. xiv, 630 pages.
2. Novaes, F.T., et al., *Lung cancer: histology, staging, treatment and survival.* J Bras Pneumol, 2008. **34**(8): p. 595-600.
3. Carvalho, L., *Reclassifying bronchial-pulmonary carcinoma: differentiating histological type in biopsies by immunohistochemistry.* Rev Port Pneumol, 2009. **15**(6): p. 1101-19.
4. da Cunha Santos, G., F.A. Shepherd, and M.S. Tsao, *EGFR mutations and lung cancer.* Annu Rev Pathol, 2011. **6**: p. 49-69.
5. Brambilla, E., et al., *The new World Health Organization classification of lung tumours.* Eur Respir J, 2001. **18**(6): p. 1059-68.
6. Xu, C., Q. Zhou, and Y.L. Wu, *Can EGFR-TKIs be used in first line treatment for advanced non-small cell lung cancer based on selection according to clinical factors? - A literature-based meta-analysis.* J Hematol Oncol, 2012. **5**: p. 62.
7. Riely, G.J., J. Marks, and W. Pao, *KRAS mutations in non-small cell lung cancer.* Proc Am Thorac Soc, 2009. **6**(2): p. 201-5.
8. Karachaliou, N., et al., *KRAS mutations in lung cancer.* Clin Lung Cancer, 2013. **14**(3): p. 205-14.
9. Lovly, C. *KRAS Mutations in Non-Small Cell Lung Cancer* 2012 [cited 2015; Available from: http://www.mycancergenome.org/content/disease/lung-cancer/kras/.
10. Shaw, A.T. *Anaplastic lymphoma kinase (ALK) fusion oncogene positive non-small cell lung cancer*. 2015.
11. Scagliotti, G.V., et al., *Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer.* J Clin Oncol, 2008. **26**(21): p. 3543-51.
12. Jemal, A., et al., *Cancer statistics, 2007.* CA Cancer J Clin, 2007. **57**(1): p. 43-66.
13. Winston, W.T. *Non-Small Cell Lung Cancer*. 2015.

14. Tam, I.Y., et al., *Distinct epidermal growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features.* Clin Cancer Res, 2006. **12**(5): p. 1647-53.
15. Pimentel, P., *RECOMENDAÇÕES NACIONAIS PARA DIAGNÓSTICO E TRATAMENTO DO CANCRO DO PULMÃO*, A.C.d. Saude, Editor. 2010: Portal da Saúde.
16. Darby, S., et al., *Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies.* BMJ, 2005. **330**(7485): p. 223.
17. Sawka, A.M., et al., *Second primary malignancy risk after radioactive iodine treatment for thyroid cancer: a systematic review and meta-analysis.* Thyroid, 2009. **19**(5): p. 451-7.
18. Rotella, V., et al., *EGFR and K-Ras mutations in women with lung adenocarcinoma: implications for treatment strategy definition.* J Exp Clin Cancer Res, 2014. **33**: p. 77.
19. Markman, M. *Genetics of Non-Small Cell Lung Cancer*. 2014.
20. Ou, S.H., et al., *Crizotinib for the treatment of ALK-rearranged non-small cell lung cancer: a success story to usher in the second decade of molecular targeted therapy in oncology.* Oncologist, 2012. **17**(11): p. 1351-75.
21. Jurmeister, P., et al., *Parallel screening for ALK, MET and ROS1 alterations in non-small cell lung cancer with implications for daily routine testing.* Lung Cancer, 2015. **87**(2): p. 122-9.
22. Sotto-Mayor, R., *[Lung cancer mortality]*. Acta Med Port, 2014. **27**(1): p. 9-11.

Annexes

ANNEX I – TNM Classification for Lung Cancer Staging

Definitions

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 visualized 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 (for example, not in the main bronchus)
- **T1a** Tumor 2 cm or less in greatest dimension
- **T1b** Tumor more than 2 cm but 3 cm or less in greatest dimension
- **T2** Tumor more than 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 more than 3 cm but 5 cm or less in greatest dimension
- **T2b** Tumor more than 5 cm but 7 cm or less in greatest dimension
- **T3** Tumor more than 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 less than 2 cm distal to the carina[1] 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, esophagus, 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 lobe, tumor with pleural nodules or malignant pleural (or pericardial) effusion
- **M1b** Distant metastasis (in extrathoracic organs)

ANATOMIC STAGE/PROGNOSTIC GROUPS			
Occult Carcinoma	TX	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1a	N0	M0
	T1b	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T2b	N0	M0
	T1a	N1	M0
	T1b	N1	M0
	T2a	N1	M0
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1a	N2	M0
	T1b	N2	M0
	T2a	N2	M0
	T2b	N2	M0
	T3	N1	M0
	T3	N2	M0
	T4	N0	M0
	T4	N1	M0
Stage IIIB	T1a	N3	M0
	T1b	N3	M0
	T2a	N3	M0
	T2b	N3	M0
	T3	N3	M0
	T4	N2	M0
	T4	N3	M0
Stage IV	Any T	Any N	M1a
	Any T	Any N	M1b

Adapted from Lung Cancer Staging, American Joint Committee on Cancer, 7h edition, 2009