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Current treatments for non-small cell lung carcinoma

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This literature review addresses the subject of non-small-cell lung carcinoma (NSCLC) and the current treatments for it. For therapeutic purposes, lung carcinomas are histologically grossly organized into two main groups that are NSCLC and small-cell lung carcinoma (SCLC). NSCLC comprises about 85% of all lung carcinoma cases and are further organized into three histologic subgroups: squamous-cell carcinoma, adenocarcinoma, and large-cell lung cancer. Smoking status and mortality rates have been widely documented to have a correlation with all lung cancers.

Diagnostical tools have improved and are sufficient to establish an accurate diagnosis without the need to rely on immunocytochemical or immunohistochemical analysis on routine basis. Despite these advanced tools and benefits of receiving early intervention, NSCLC is often diagnosed late, which precedes poor prognosis.

Lung cancer develops over long period of time and often presents itself with non-specific respiratory symptoms, such as coughing, shortness of breath and sometimes bloody sputum. With smokers already excessively represented in pulmonary diseases and simultaneously being the dominant bracket to develop NSCLC, some of the warning signs of this cancer might go unnoticed for longer period amongst them.

Current treatment options include traditional cancer treatments such as surgery, radiotherapy, and chemotherapy. Emphasis on these has remained strong, but treatments have begun convert into new and more specific options. Combination therapies, targeted therapies and immunotherapies are increasingly used in late-stage disease. Lastly, we touch on the possibilities of novel bacteria-based therapies for lung cancer.

Keywords: non-small-cell lung cancer, surgery, radiotherapy, chemotherapy, immunotherapy, targeted therapy, bacteria-based therapy

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1 Non-Small-Cell Lung cancer

1.1 Overview

During the 19th century, lung cancer in general and particularly primary lung cancer quickly rose from being a rare incidence to now leading the mortality charts among cancers worldwide. This disease has traditionally been more prevalent among the more developed countries for several reasons, which include early industrialization and subsequent deterioration of air quality, and the accumulation of wealth and the subsequent exposure to tobacco, the biggest risk factor of lung cancer. (Didkowska, et al., 2016) (1)

Developed nations saw their golden age of tobacco consumption in the dawn of the 20th century and since peaking in the middle century, the incidence trend for the male population has leveled off, even reversed. Apart from a few countries, the incidence rate will continue to rise. This phenomenon can be attributed to several causes, including aging of population, particularly in wealthier countries, total increase in tobacco consumption and increased life expectancy. However, contributing to the latter notions, the focus of incidence will likely turn more towards developing nations. Annual deaths are expected to rise above 3 million until 2035, with total deaths also rising in developed countries despite the recent years decline in smoking in men and plateau in women. (Didkowska, et al., 2016)

Carcinomas of the lung are a heterogenous set of diseases and from a histological perspective they are broadly divided into two categories, SCLC and NSCLC, of which the latter accounts for 85% of the cases and will be the focal point of this review. NSCLC can be divided into groups of histological subtypes of which the two most prevalent are lung adenocarcinoma (LUAD) and lung squamous-cell carcinoma (LUSC). This categorization not only differentiates these diseases by histology, but subtypes also differ in their tendency to etiological causes and mutational burden, which in return drastically changes the approach to treating them now and in the future. (Herbst, et al., 2018)

Smoking is heavily associated with LUSC, while LUAD is the most common subtype among the never-smokers, a group which has many variations in the literature, but we decide to use the definition for people who have exposed themselves to less than 100 cigarettes during their lifetime, while everyone else will be referred as ever-smokers. LUAD in never-smokers has

been shown to have association with exposure to environmental pollutions, second-hand smoking, and genetic susceptibility. It also appears to have higher prevalence amongst women, particularly in Asia. (Sun, et al., 2007)

Concerted efforts to curb lung cancer must be focused on primary prevention. Secondary prevention is not a desirable option even for the wealthier countries because of the high cost of treatment. For the time being, tobacco control seems to be the only serious and most effective global strategy to combat lung cancer, although wealthy countries might benefit from the implementation of better and more accurate screening methods in the future. (Didkowska, et al., 2016)

Combating tobacco and producing better primary screening is only the foundation for the solution, and new diagnostical tools, further subtyping, and targeted therapies are under development to improve clinical outcomes for the patients. Even though progress has been steadfast for the past 20 years and we have witnessed the advent of targeted therapies and immunotherapies, lung cancer still proves to be evasive and expensive medicine has only yielded mere months to overall survival (OS), thus providing only a partial remedy to the problem. Further research of the mechanisms of tumor resistance to targeted therapy and better predictors of effectiveness of a given drug is needed to make sound decisions to concentrate the right therapies to the right populations that are likely to benefit from them. (Herbst, et al., 2018)

Lung cancer remains deadly across the world. Reviewed survival data of some 5,3 million patients found the age-standardized 5-year net survival to generally stand in the range of 10-20% with only modest variability across different regions. (Allemani, et al., 2015)

Despite the telling numbers in terms of incidence, mortality, and urgent need of improved treatments, lung cancer research has long gone underfunded (Dela Cruz, et al., 2011). Interestingly, major funding has instead been funneled into less burdening cancer niches, such as leukemia. (Carter & Nguyen, 2012)

1.2 Risk factors

1.2.1 Tobacco

First notions of cause-effect of tobacco consumption and lung cancer were case reports published in the 1930s, but it was only after two landmark studies published in 1950 that stood out with sufficient size, high response rates and clearly defined categories of smoking that serious research in this topic would gain interest (Doll & Hill, 1950, Wynder & Graham, 1950). Initial resistance was attributed to public and academic denial, for majority of male public and even physicians in the era were regular smokers (Thun, 2010).

After 70 years of piling evidence, cigarette's role in cancer development has been confirmed numerous times and since the inception of this notion, at least 50 carcinogens in tobacco fumes have been identified (Smith, et al., 2000a, Smith, et al., 2000b).

Carcinogens contained in tobacco are associated with activating Kirsten Rat Sarcoma (*KRAS*) gene as well as inhibiting the tumor protein P53 (*TP53*) gene. Some of these mutations persist through time, which may partly explain the incidence of cancer years after smoking cessation in an individual. (Dela Cruz, et al., 2011) Smoking increases the relative risk 10- to 30-fold compared with lifetime never-smokers (Mattson, et al., 1987). However, while smoking increases the likelihood of cancer to such extends, only one in 9 smokers eventually develop the disease, suggesting wider genetical susceptibility is probably included in the equation (Jemal, et al., 2005).

1.2.2 Never-Smokers

Never-smokers constitute a quarter of lung carcinoma cases, and as an independent subset represent the 7th most common cause of cancer deaths in the world. It is disproportionately more common in women, but geographical variability has also been shown, placing the emphasis in Asia. Plenty of unique attributes differentiates it from the lung cancer occurring in ever-smokers. Cancers arising in never-smokers tend to occur more frequently in the distal airways and present LUAD histology. Molecular epidemiologic studies suggest different mutational tendency as opposed to cancer in ever-smokers. These different molecular patterns make lung cancer in never-smokers more vulnerable to certain targeted therapies. (Sun, et al., 2007)

Relative importance of various risk factors, such as radon exposure in homes, second-hand smoke, such as combustion, have not been clearly defined worldwide. Overall lung cancer in never-smokers differs from that in ever-smokers in terms of tumor biology, prognosis, and response to therapy, but more research in this field is needed. (Rudin, et al., 2009)

1.2.3 Other

Fine particles with diameter of 2.5μm or less ($PM_{2.5}$) have been shown to increase risk for lung cancer when continuous chronic exposure is present. In a 26-year follow-up study, every 10μg/m^3 increase in $PM_{2.5}$ concentration was associated with 15-27% increase in lung cancer mortality. (Turner, et al., 2011)

Cancer susceptibility is partly determined by genetic factors, although the risk is mostly realized if person is an ever-smoker. Other risk factors include pulmonary fibrosis, human immunodeficiency virus infection and alcohol. (Duma, et al., 2019) One meta-analysis comprising of 32 studies estimated that having a family history of lung cancer doubles the risk. Elevated risk was also seen in never-smokers. (Matakidou, et al., 2005)

Estimates show that 10% of men and 5% of women lung cancer deaths can be connected to 8 carcinogens that classify as occupational exposure, they are asbestos, arsenic, beryllium, cadmium, chromium, nickel, silica, and diesel fumes. Ionizing radiation has also been proved to be carcinogenic factor. Other factors such as gender, age and ethnicity also affect one's risk, but will not be covered in this review. (Dela Cruz, et al., 2011)

1.3 Diagnosis & classification

One of the biggest challenges in dealing with lung cancer and adding to mortality and financial burden, is the elusiveness of the disease in its initial stage. Gradual, but slow progression and non-specific symptoms make all the difference, for majority of lung cancers commonly presents with little to no symptoms. Often the tumor is found at an advanced stage, which significantly lower the prognosis. (Mäkitaro, 2007)

Symptoms are most commonly sign of late-stage disease and in their own remain highly unspecific to this disease. Different presentations derive from several causes. The obstruction of normal anatomy in the lungs due to primary tumor can cause cough, bloody sputum, recurrent

pneumonia, shortness of breath, atelectasis, and stridor. Dyspnea can be caused by a tumor growing near phrenic nerve supplying the diaphragm. Mediastinal growth can damage the recurrent nerve, causing voice hoarseness or problems in swallowing. Other novel presentations include vena cava syndrome, shoulder-arm pain, Horner's syndrome, and bone destruction. (Mäkitaro, 2007)

Sometimes first signs are the ones attributed to metastases affecting other organs. Metastases in the liver can cause abdominal pain and elevation in liver enzymes, albeit this usually requires the tumor to have grown radically in size. Neurological symptoms such as headache, vertigo, nausea, seizures, or deficits in sensory or motoric capabilities might be evident if central nervous system is affected. Metastases in the bone can cause pain or pathological fractures. Despite all the possible organ-specific symptoms, sometimes only non-specific constitutional symptoms, such as fever, decreased appetite, weight-loss or malaise are the only clues present. Furthermore, an abnormal chest radiograph, accompanied with or without symptoms, can make a suspect and proceed to further evaluation. (Mäkitaro, 2007, Rivera, et al., 2013).

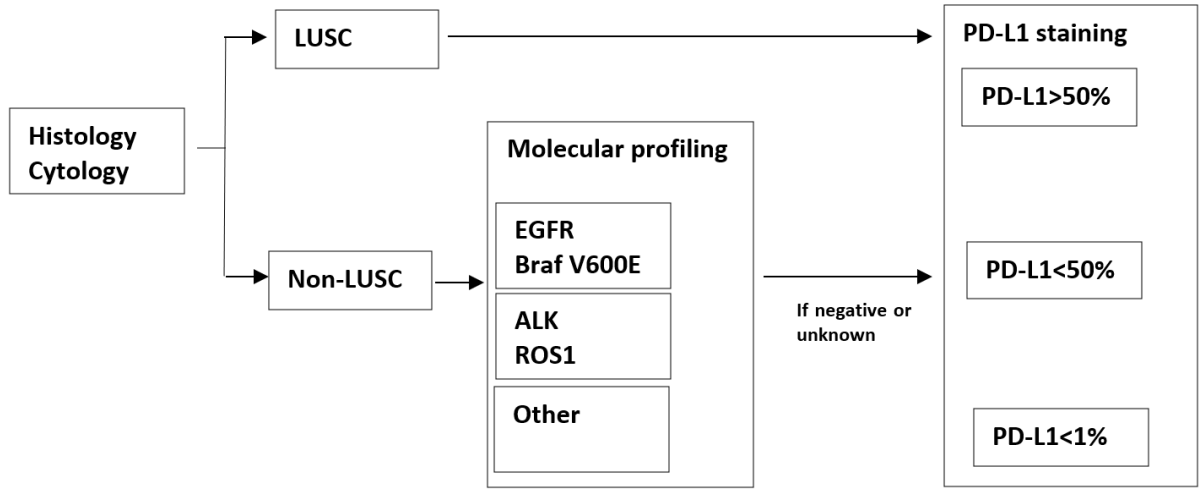
Diagnosis will be confirmed through biopsy. Two kinds of biopsies are tissue biopsy, which are obtained by either bronchoscopy or surgical means, while cytological samples can be acquired from effusion, aspiration, or by brushing. Morphologic evaluation is conducted next as it will guide the recognition towards diagnosis. Histological samples are stained with hematoxylin and eosin while cytological samples use giemsa staining. Sometimes clear characteristics might yield the immediate recognition as either LUAD or LUSC and further immunocytochemical or immunohistochemical analysis is not needed. Should neuroendocrine features be present, sample can be classified as either SCLC or NSCLC. If there is no clear characteristics of LUAD or LUSC, the sample will be classified as NSCLC not otherwise specified (NOS). (Reck & Rabe, 2017)

Further subdivision is accomplished by using immunocytochemical or immunohistochemical analysis, mucin staining, or molecular data. Classification for NSCLC favoring LUAD is determined when sample tests positive for sarcolectin and NK2 homeobox 1, additionally LUSC markers must be negative. On the contrary, if sample tests positive for one or more LUSC leaning markers, such as p63, sex determining region Y-box 2, cytokeratin 5, or cytokeratin 6 and negative for LUAD markers, NSCLC favoring LUSC is diagnosed. If none of the forementioned markers are positive, the sample is classified as NSCLC NOS. (Reck & Rabe, 2017)

One subtype of NSCLC includes large cell carcinoma, which is diagnosed by excluding LUSC and LUAD by histology and their specific markers. The literature is not decisive whether large cell carcinomas are distinct from LUSC or LUAD (Chen, et al., 2014, Pelosi, et al., 2015).

Once diagnosis is established, further classification with molecular testing is recommended for those with non-LUSC NSCLC. The need for this approach is becoming ever more important as the number of treatable oncogenic alterations continues to expand. The list of potential treatable alterations continues to expand, but some of the most common articles to screen include mutations in the genes encoding epidermal growth factor receptor (*EGFR*), v-Raf murine sarcoma viral oncogene homolog B (*BRAF*), anaplastic lymphoma kinase (*ALK*) and proto-oncogene tyrosine-protein kinase ROS (*ROS1*) genes. More recent example is assessing the expression of programmed death-ligand 1 (PD-L1), this marker is also assessed in LUSC. Algorithm for diagnostics of NSCLC is depicted below (Figure 1). (Reck & Rabe, 2017)

Figure 1: Diagnostic algorithm for NSCLC



The staging and histology of a tumor defines the treatment and prognosis of a given patient. The TNM Classification of Malignant Tumors (TNM), provided by the Union for International Cancer Control, is the international standard for tumor staging. Having its latest updates released in 2017, the current volume marks the 8th edition of its kind (Table 1). The section regarding lung cancer is maintained by the International Association for the Study of Lung Cancer. In the wake of personalized medicine, correct staging is at the very core of treatment. Correctly done analysis of the tumor will guide the therapy and monitor its effectiveness. (Detterbeck, et al., 2017)

Table 1: Staging of lung carcinoma in relation to TNM status

	N0	**N1**	**N2**	**N3**
T1	IA	IIB	IIIA	IIIB
T2a	IB	IIB	IIIA	IIIB
T2b	IIA	IIB	IIIA	IIIB
T3	IIB	IIIA	IIIB	IIIC
T4	IIIA	IIIA	IIIB	IIIC
M1a	IVA	IVA	IVA	IVA
M1b	IVA	IVA	IVA	IVA
M1c	IVB	IVB	IVB	IVB

Initial estimation of tumor severity is clinical staging, which is accomplished by imaging. Computed tomography (CT) is considered the standard tool for staging. However, advancements in technology, namely positron emission tomography (PET) and magnetic resonance imaging can offer improved accuracy in staging-phase. Utilizing fluorodeoxyglucose (FDG) in PET-CT has been shown to be better at assessing nodal status and disease than a regular contrast enhanced CT scan. FDG PET-CT scan can also determine occult carcinomas in the bone better than bone scan, as before changing the mineral composition of the cortical bone, new metastases initially affect the bone marrow, which is something the bone scan is unable to detect. (Reck & Rabe, 2017, Akhurst, 2018)

Clinical staging alone is not sufficient for diagnosis. The ability of CT to detect mediastinal lymph node metastasis yielded approximately 55% sensitivity and 81% specificity. For PET-scans, the corresponding values were a little better, 77% sensitivity and 86% specificity. To limit the number of futile surgeries, which often occur as result of underestimated staging, advanced staging must be performed through histological evaluation of biopsies. (Silvestri, et al., 2013)

1.4 Pathogenesis

Widespread analyses have defined NSCLC as a set of distinct diseases that carry substantial heterogeneity in terms of cellular and genetic property. Introduction of advanced methods for gathering genomic information, such as next-generation sequencing, have enabled a deeper look into the genomic alterations that precede NSCLC and have subsequently revealed novel therapeutic targets. Advanced genetic methods have also revealed the complexity of these tumors as they possess alterations not only in traditional protein kinases but also epigenetic modifiers, transcription factors, splicing factors and genes involved in modifying immunological response to tumors. The two main forms of NSCLC, LUSC and LUAD, have different tendencies to their pathophysiology that are reflected in their genetic and histological appearance. (Chen, et al., 2014)

LUSC generally emerges from the proximal airways and its histological differentiation into squamous cell resemblances pseudostratified columnar epithelium that is lining nearby anatomical sites, namely the upper airways and tracheal area. Genetic disparities are evident, but some are more modest than others as many genetic alterations share a close frequency in both subtypes, few examples of this are *TP53* and receptor tyrosine kinase gene encoding neurofibromin 1. Genes that have shown mutations and preference towards LUSC include fibroblast growth factor receptor 1 (*FGFR1*), *FGFR2*, *FGFR3*, cluster of differentiation 167b, phosphoinositide 3-kinase and phosphatase and tensin homolog. However, the number of genetic alterations specific to LUSC is smaller compared to LUAD. (Chen, et al., 2014)

LUAD typically arises in the more distal airways and the histology is often glandular. Genetical alterations that have greater recurrence in LUAD include *KRAS*, *EGFR*, but also fusion oncogenes such as *ALK* and the *ROS1* receptor tyrosine kinase. The incidence of mutations is 5-6 times smaller in the never-smoking population. (Chen, et al., 2014)

Interaction with the immediate microenvironment surrounding the tumor is vital in the tumorigenesis of all cancers. The relationship between these two differ by tumor type, as sometimes adapting the microenvironment helps stabilize the growth, and in others stands to block further growth or even favor tumor degradation. Lung cancers typically aim to promote local angiogenic action as increasing flow of nutrients is essential to their growth. Platelet-derived growth

factor and vascular endothelial growth factor (VEGF) are some of the signal proteins secreted by tumors to induce angiogenesis. Attraction and increased traffic of white blood cells of both myeloid and lymphoid lineages benefits the tumor. The mechanism for this effect remains vague, but it is believed leucocytes ability to degrade the matrix of microenvironment potentiates tumor progression. Leucocytes also appear to promote tumor cell proliferation, metastases, and angiogenesis. Tumors alone can evade the natural immunological processes by displaying immune receptor programmed cell death protein 1 (PD-1). (Chen, et al., 2014)

1.5 Prognosis

In contrast with the positive progress of other cancers, the outlook for lung cancer remains relatively modest. The age standardized 5-year net survival percentage remains generally lower than 20% depending on geographical location. This is largely attributed to tumors being diagnosed at a later stage, which is more often a predictor of bad outcome. (Allemani, et al., 2015)

The United States National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER) has tracked nations cancer statistics regarding NSCLC from 1975 to 2017. General prognosis for men and women has always held a gap of 6-10% favoring women. Racial discrepancy in the US favors whites over blacks, and the current 3-6% gap appears to be widening every year. However, improvements in diagnostics and treatment have had some positive impact as overall 5-year relative survival has climbed from around 16% to around 26% in the past 40 years. (Noone, et al., 2018)

Greatest impact for prognosis by far comes from the stage at which the cancer is diagnosed. Females tend to have their cancers diagnosed earlier than men. Discovering the tumor at a localized stage offers over 60% chance of 5-year relative survival, but that number drops significantly if the tumor has metastasized, yielding only around 7% survival rate. Data from SEER reveals mismatch between late diagnosis and 5-year relative survival (Table 2), which drags the overall prognosis of NSCLC down, highlighting the importance of early diagnosis. (Noone, et al., 2018)

Table 2: Stage distribution (%) versus 5-year Relative Survival (%) 2008-2014 by Stage at Diagnosis

Stage at Diagnosis	Both Sexes	Males	Females
Localized	19 vs 60.1	17 vs 53.7	22 vs 65.6
Regional	24 vs 33.4	24 vs 29	23 vs 38.4
Distant	55 vs 5.5	57 vs 4.5	53 vs 6.7
Unstaged/Unknown	2 vs 13.8	2 vs 12.9	22 vs 14.9

Young people (age <40) have been shown to have more NSCLC that carry genomic alterations that are targets for oncogenic treatments, but prognosis in these cases is as bad as with old patients (aged 70+), interestingly, the age groups in between that carry these treatable oncogenic alterations have better survival rate. The reason for this remains unclear, but one hypothesis suggests that NSCLC harboring certain mutations in younger people might be more aggressive than the ones present in older populations. Younger age in general is associated with considerably better prognosis. People younger than 45 have 5-year relative survival of 38.7%, while for people older than 75 that number is 20.8%. Partial explanation might be the accumulation of poorly treatable LUSC in older people, as the effects of smoking begin to weigh. (Sacher, et al., 2016, Noone, et al., 2018)

Survivors of NSCLC are suggested to have an intense follow-up period involving general examination every 3-6 months and a low-dose axial CT scan every 6 months for 1-2 years, followed by annual checks and CT-scans for years 3-5+ afterwards. (Shapiro, 2018)

2 Current Treatments

2.1 Surgery

Surgery is the primary treatment for patients with NSCLC clinically staged I, II and some selected IIIA disease. The challenge of surgery lays with balancing the adequate resection of the tumor, while preserving the functionality of the remaining lung tissue (Zappa & Mousa, 2016). The standard procedure for surgical candidates is lobectomy by video-assisted thoracoscopic surgery (VATS) (Gridelli, et al., 2015).

Adjuvant therapy is commonly used to prolong survival and reduce the risk of relapse. Options for adjuvant therapy include radiation therapy, chemotherapy, and targeted therapy. Stages IIA, IIB and IIIA are usually treated with postoperative chemotherapy to prolong survival. (Zappa & Mousa, 2016) One meta-analysis based on 34 trials comparing surgery plus chemotherapy versus surgery alone showed increased survival of 4% at 5 years (95% confidence interval (CI) 3-6). Second meta-analysis based on 13 trials comparing surgery plus radiotherapy and chemotherapy versus surgery plus radiotherapy also showed estimated 4% increase in 5-year survival (95% CI 1-8), reinforcing the beneficial effect of adjuvant chemotherapy. (Burdett, et al., 2015)

Several techniques have been developed to achieve resection, each suited better for different kinds of situation. Lobectomy is generally regarded the primary surgical procedure for lung cancer. The procedure includes the resection of a single lobe and adjacent lymph nodes. As compared to more radical resections, lobectomy has better potential for preserving pulmonary function. Mortality for lobectomy is 2-4%. A variation of this technique is the sleeve lobectomy, in which a part of the common airway is removed along with the resection of the lobe. Airway reconstruction, accomplished either by using intercostal muscles or by pleural wrapping of the anastomosis, concludes the operation. As a more complicated variant to traditional method, the sleeve lobectomy carries slightly higher post-operative burden, mortality and morbidity range from 2% to 11%. Sleeve lobectomy is used as an alternative to pneumectomy in a subset of patients with bronchogenic carcinoma. (Rotman, et al., 2015)

Pneumectomy removes an entire lung along with its lymph nodes. This radical procedure might be indicated when larger areas of the lung or bronchi are affected. Modifications of this surgery include intrapleural pneumectomy, in which the visceral pleura is also removed, and extrapleu-

ral pneumectomy, which involves resection of the lung, pleurae, and even the proximal pericardium and hemidiaphragm. Mortality for pneumectomy is 6-8%. (Rotman, et al., 2015)

In wedge resection, the tumor and some margin of healthy proximal parenchyma is removed. The resected area is small, and non-anatomical boundaries are used. Small tumors, metastatic lesions, lung biopsies and early-stage cancers in the elderly are included in the list of indications for this procedure. Being minimally invasive in nature, this technique is associated with lesser rates of complications. (Rotman, et al., 2015) The overall 5-year survival rate hovers near the outcomes of other surgical techniques, but radically drops to being inferior if indication is conservative surgery for patient with many co-morbidities. Wedge resection should only be performed on carefully selected patients with adequate indication. (Nakamura, et al., 2011)

Wedge resection is often performed utilizing VATS or robot-assisted thoracoscopic surgery. Meta-analysis of 21 studies concluded that VATS has no statistical difference to open lobectomy in terms of post-operative complications or locoregional tumor recurrence, but systemic recurrence rate was lower (risk ratio (RR)=0.57; 95% CI, 0.34-0.95; p=0.03) and 5-year survival rate was improved (RR=0.72; 95% CI 0.45-0.97; p=0.04). Some of these studies were not randomized. Patient selection was also biased, possibly influencing the outcomes. (Yan, et al., 2009)

An alternative to lobectomy, segmentectomy can be performed to a selected group of patients. This method resects larger margins and more lymph nodes compared to wedge resection, but spares tissue in comparison to traditional lobectomy. As in wedge resection, VATS can be utilized in this method to yield better outcomes and shorter recovery times compared to thoracotomy. (Rotman, et al., 2015)

The topic of lymph node dissection has not been settled in literature and while sometimes lymph nodal evaluation is done by visual inspection of the unopened mediastinum, others opt on surgical approach. Two methods of surgical intervention are used to evaluate lymph node involvement in lung cancer. Sampling means the removal of one or more lymph nodes, which are selected based on pre-operative or intra-operative findings. Systematic or full nodal dissection means the removal of all lymph nodes including the neighboring mediastinal tissue within anatomically defined area. (Koulaxouzidis, et al., 2013)

The European Society of Thoracic Surgeons recommended in their guidelines (2006) that achieving total resection requires systematic lymph node dissection. For a peripheral squamous T1-tumor, selective nodal dissection is acceptable, as the lymphatic drainage in the area creates low-risk environment for unforeseen N2 disease. (Lardinois, et al., 2006)

One meta-analysis based on 3 studies also concluded that in patients with cancer stages I to IIIa, mediastinal lymph node dissection appeared to improve survival rates compared with mediastinal lymph node sampling (hazard ratio (HR) 0.63, 95% CI 0.51-0.78). (Manser, et al., 2005)

Advances in surgery have reduced the invasiveness, making the surgery less prone to complications. Japanese Lung Cancer Registry study data shows that the death rate within 30 days after resection has decreased from 1.4% in 1994 to 0.4% in 2004. (Sawabata, et al., 2011)

The US National Cancer Institute have constituted the Common Terminology Criteria for Adverse Effects. The criteria range from grade 1 to grade 5 which, in ascending order, are mild, moderate, severe, life-threatening or death. (NCI, 2019)

Post-surgical complications are divided into two groups, immediate- and late complications. The diagnosis is heavily dependent on chest x-rays and computed topography, as many complications have a distinct radiographic pattern. Immediate complications take place during the days 0-30 postoperatively. The most common of these include atelectasis of the lung, pneumonia, empyema, and prolonged air leak. Some of the rarer complications include lung torsion, oedema, and cardiac herniation. Pneumonia is the leading cause of postoperative mortality, it affects 2-22% of the patients and has mortality rate of up to 25%. Other serious complications are bronchopleural fistula, pulmonary edema (50-100% mortality) and acute respiratory syndrome (80% mortality). Late complications appear after the 30-day mark postoperatively. These include bronchial anastomotic stenosis, late bronchopleural fistula, late onset empyema, esophagopleural fistula and postpneumectomy syndrome. (Rotman, et al., 2015)

Tumor recurrence can be regarded as a form of long-term complication. Locoregional tumor recurrence presents as regional tumor in mediastinal wall, parietal pleura, surgical margins or as metastases in the mediastinal lymph nodes. Rate of recurrence in a 5-year period is 20-30% for stage I cancer, 50% for stage II and 70-80% for stage III. (Rotman, et al., 2015)

Pulmonary impairment due to pre-existing illness such as chronic obstructive pulmonary disease (COPD) can dramatically alter the operability of a patient. Almost 37% of patients that have been diagnosed with lung cancer and are suitable for anatomical resection are deemed inoperable due to severe pulmonary function impairment. For these patients, the rates for surgical morbidity and mortality are 83% and 33% respectively. Rehabilitation before surgery with physical training has been hypothesized to improve survivability, as it is a known to reduce post-operative morbidity and mortality of colorectal, heart and spinal surgery. The oncological timing for lung cancer, however, does not allow for long endurance training periods and shorter periods of more intensive training has been proposed. No clear statistical evidence has yet been reported, but COPD patients probably benefit from preoperative rehabilitation training. (Sanchez-Lorente, et al., 2018)

2.2 Radiotherapy

Radiotherapy can be applied externally or internally. Internal radiotherapy is preferred, if radiation source can be placed near the tumor and is therefore mainly used for treating cancers in gynecological areas, anorectal area, or the eyes. External radiotherapy is used for most cancers. It utilizes distant source, such as x-ray machines, proton- or neutron beam machines to deliver radiation. The unit for dose is Gray (Gy), which is defined as one joule of radiation energy per kilogram of matter. The two most common methods for external radiation are external beam radiotherapy (ERBT) and stereotactic body radiation therapy (SBRT). (Noone, et al., 2018)

Radiotherapy can be used in all stages of lung cancer and while sometimes used for palliative care, extensive and ongoing advancement of technologies and research have enabled the use of radiotherapy increasingly as curative option, particularly when combined with systemic therapy or surgery. (Brown, et al., 2019)

Surgery persists as the primary treatment for early stage (stage I/II) NSCLC. However, sometimes pre-existing conditions (e.g., COPD or severe cardiovascular disease) cause the patient to be classified as “medically inoperable”. Patients who refuse surgery are also classified as such, and radiotherapy is subsequently offered as an alternative. (Abel, et al., 2019) SBRT has become a viable alternative to surgery largely owing to its efficacy, convenience, and acceptable toxicity profile (Kann, et al., 2019). A large study using univariable and multivariable analyses comprising of 15,110 subjects that had early-stage NSCLC found the median OS to be 44

months for LUAD and 33 months for LUSC ($p<0.0001$). OS at predetermined time intervals were 85% versus 83% at 1 year, 58% versus 43% at 3 years and 36% versus 24% at five years for patients diagnosed with LUAD and LUSC, respectively ($p<0.0001$). Improved survival was associated with female sex, African American race, T1 lesions, age <75 and LUAD histology. (Abel, et al., 2019) Patient selection inherently makes the comparison between surgery and radiotherapy difficult, as radiotherapy is often only considered after the patient is considered in-operable. Analysis comparing the outcomes of 143 patients treated with surgery against 197 patients treated with SBRT found no difference in OS (HR 1.07; 95% CI 0.74-1.54; $p=0.73$), when prognostic covariables were adjusted for. (van den Berg, et al., 2015) One review showed similar 2-year OS after SBRT (70%, 95% CI; 67-72%) and surgery (68%, 95% CI; 66-70) for stage I patients (Soldà, et al., 2013).

The treatment for inoperable locally advanced (stage III) NSCLC has changed very little during the past 30 years. The standard is concurred chemoradiotherapy and although high dose of radiation delivered to the tumor is associated with higher tumor resolution, this effect has not been observed when applying dose escalation to the standard treatment. (Brown, et al., 2019) On the contrary, one randomized Phase III trial (RTOG 0617) with subjects having stage IIIA/B disease compared 60Gy and 74Gy radiotherapy (with or without cetuximab), the median survival for patients with higher dose was 20.3 months versus 28.7 months for the standard dose. This gap was partly explained by the increased toxicity and complications related with higher doses. (Bradley, et al., 2015) Median survival for stage III NSCLC treated with radiotherapy has improved significantly over the last 20 years. Better staging and advanced preoperative imaging deserve some credit, but the increased survival is mostly attributed to the addition of immunotherapy. (Brown, et al., 2019) One year of durvalumab following radiotherapy increased the 2-year OS (66.3% with durvalumab versus 55.6 with placebo). Progression-free survival (PFS) was also increased (median, 28.3 months versus 16.2 months). (Antonia, et al., 2018) Concurrent chemoradiotherapy is more effective treatment to radiotherapy alone as will be discussed later (O'Rourke, et al., 2010).

Palliative radiotherapy is used in metastatic (stage IV) NSCLC. The objective is to decrease symptoms such as pain, cough, and hemoptysis. New interest in treating oligometastatic NSCLC has surfaced, as several studies have demonstrated radiotherapy to have positive effect on the OS, with patients granted several months of median survival as opposed to standard maintenance therapy. However, these studies are not sufficient to draw final conclusions and

phase III evidence is still required. Many studies are currently examining the use of radiotherapy in metastatic NSCLC. (Brown, et al., 2019)

ERBT involves delivering relatively small doses of radiation (1.8-2Gy per fraction) to the tumor via two-dimensional beams using a linear accelerator. Planning of the procedure does not involve 3D-imaging by CT, as it can instead be accomplished with diagnostic X-ray machine and the treatment is guided based on these images. Detailed anatomy of the tumor is lacking and therefore dose absorbed into the healthy tissue is greater than in more advanced techniques. Outcomes are disappointing and SBRT has replaces EBRT as the primary radiation therapy. (Parashar, et al., 2013)

SBRT utilizes multiple small, accurate and highly focused external beams of radiation to conduct large doses (e.g., 20Gy per fraction) on the tumor. Treatment is performed during several sessions over a few weeks. The method is noninvasive and guided by advanced imaging, which allows well-defined small tumors to be treated. Indications for the use of SBRT includes tumors less than 5 cm in diameter in which lymph nodes are negative, tumors should also have a distance minimum of 2 cm from the tracheobronchial tree, as proximity to central areas increases the risk of complications. SBRT can be used extracranially. (Timmerman, et al., 2010) Multiple techniques can be used to deliver SBRT treatments, such as using a conventional linear accelerator in fixed field mode or dynamically with intensity modulated fixed field mode. (Parashar, et al., 2013, van den Berg, et al., 2015)

SBRT is associated with excellent local disease control. Challenges for this method are regional and distant failures, which often lead to increased morbidity and mortality. Recurrence occurs in 20-30% of patients. There is also a significant risk of clinically occult nodal metastases, with increased risk related to tumor size. The primary pattern of failure is regional-distant, but the risk can be reduced by combining SBRT with systemic-therapy. Local recurrence is significantly higher in LUSC as opposed to LUAD histology, partly explaining the difference in survival. (Kann, et al., 2019) Locoregional failure is also more prevalent in SBRT than in surgery (van den Berg, et al., 2015).

Technological advancements have allowed the tumor mass to be better distinguished from the healthy tissue and subsequently higher doses of radiation can be used to deliver better results. Still, normal tissue cannot be completely separated from the radiated area, which is why the

topic of complications stays relevant and must always be considered before the treatment. Post-radiation therapy complications arise locally as healthy tissues are damaged. Adjacent structures include the healthy lung, pleura, heart, great vessels, thymus, lymph nodes, esophagus, liver, breast, and bones. Complications (Table 3) are divided into early- and late complications. Early complications are manifested in the following weeks or months after the radiotherapy. Late complications typically manifest after several months or even years. (Baker, et al., 2016, Benveniste, et al., 2019)

Given the low prognosis for untreated NSCLC, there are no absolute contraindications for radiotherapy. Active interstitial lung disease may be an important risk factor for severe toxicity and is considered a relative contraindication. (Shultz, et al., 2015) Toxicity and the risk for complications is also increased for central tumors, which are located within two centimeters in all directions of any vital anatomy in the mediastinum (Baker, et al., 2016).

Table 3: Post-Radiation Therapy Complications

Anatomic site	**Early complications(s)**	**Late complication(s)**
Lungs	Infection, organizing pneumonia	Tumor recurrence
Pleura	Pleural effusion	…
Heart	Pericardial effusion	Coronary artery disease, valvular disease, cardiomyopathy, chronic pericarditis
Great vessels	Vascular stenosis	Vascular wall calcifications, vascular occlusion, pseudoaneurysm
Thymus	Simple cysts	…
Lymph nodes	…	Calcified lymph nodes, fibrotic mass
Esophagus	Ulceration, esophageal dysmotility, perforation, fistula	Stricture, ulceration, fistula
Liver	Focal hepatitis	Atrophic liver changes
Breast	Diffuse skin thickening	Fat necrosis, dystrophic calcification, skin retraction
Bones	Edema and osteopenia	Pathologic fractures, osteochondromas and osteoradionecrosis.
Malignancy	Local or distal recurrence	Radiation-induced thoracic malignancies (e.g., sarcoma, breast, bone and lung cancer)

At 5 years endpoint, patients with early-stage NSCLC treated with SBRT have a risk for local or distant recurrence of 9-20% and 20%, respectively. Locally advanced NSCLC have local and distant recurrence of 30-40% and 40-50%, respectively. (Baker, et al., 2016)

2.3 Chemotherapy

Chemotherapy has a broad spectrum of indications in the treatment of NSCLC. Chemotherapy is used as an adjuvant therapy in stages II/IIIA NSCLC in combination with surgical resection to enhance survival, for chemotherapy can address the possible dissemination of tumor cells and distant spread, something the local intervention fails to do. Neoadjuvant chemotherapy has the rationale of attenuating the central tumor prior to surgery, but studies have shown it to have no benefits and is therefore not recommended. For the unresectable stage III NSCLC, concurrent chemoradiation therapy is preferred, but the 5-year prognosis remains poor, as only 15% of patients are alive. Stage IV NSCLC requires systemic treatment and platinum doublet chemotherapy has traditionally been the standard. However, the advent of immunotherapy has changed the treatment of metastatic NSCLC as will be discussed later. (Duma, et al., 2019)

Lung Adjuvant Cisplatin Evaluation (LACE) comprised analysis of five large trials (4584 patients) in which patients had underwent complete resection and had received adjuvant cisplatin-based chemotherapy. Several drugs used to complement the platinum base included etoposide, vinorelbine and vina-alkaloid. However, no statistically relevant difference between their efficacy was observed. The results showed a 5-year absolute benefit of 5.4% (HR of death was 0.89, 95% CL, 0.82 to 0.96, p=0.005). For stage IA NSCLC chemotherapy showed to increase the risk of death (HR of 1.40, 95% CI 0.95-2.06). Stage IB remains controversial and might be beneficial to a high-risk individual. LACE study showed only mildly improved benefit for IB disease (HR 0.93, 95% 0.78-1.10). Stage II demonstrated a clear benefit (HR 0.83, 95% CI, 0.73-0.95) as did stage III NSCLC (HR 0.83, 95% CI, 0.72-0.94). (Pignon, et al., 2008)

Adjuvant chemotherapy is also beneficial in patients (stage II-IIIA NSCLC) treated with surgery or surgery plus radiotherapy as discussed earlier. The addition of chemotherapy corresponded to a 4 percent increase in OS at 5 years (33 versus 29 percent). (Burdett, et al., 2015)

Concurrent chemoradiotherapy is the standard for unresectable stage III NSCLC. The optimal choice of chemo agent remains unclear. A multicenter randomized phase III trial found the

combination of etoposide/cisplatin (EP) to be superior against carboplatin/paclitaxel (PC) (3-year estimated difference in OS was 15.0%, p=0.024), median survivals were 23.3 months and 20.7 months, respectively. Pneumonitis (grade 2 or higher) was more common with PC and esophagitis (grade 3 or higher) was more common with EP. (Liang, et al., 2017)

For decades, the treatment of advanced NSCLC has heavily relied on platinum-based cytotoxic chemotherapy. Although new agents and novel combinations have been tested in many trials, non-platinum combinations have not shown superior results. (Lwin, et al., 2013) Whilst poor outcome for advanced NSCLC is inevitable, chemotherapy improves OS in all patients when compared to supportive care. Meta-analysis and systematic review of 16 randomized controlled trials (RCTs) concluded that chemotherapy produced significant benefit (HR, 0.77; 95% CI, 0.71-0.83; p<0.0001), which translates to absolute improvement in survival of 9% at 12 months. This analysis concluded that the drug of choice, or whether single agents or combination was used, had no effect. (NSCLC Meta-Analyses Collaborative Group, 2008)

The discovery of treatable oncogenic-driver pathways has transformed the research of advanced NSCLC into a swiftly evolving platform, as new treatments are being implemented for selected patients. Most patients, however, do not harbor actionable driver mutations and chemotherapy is the primary treatment for them. For all categories of NSCLC without *EGFR* mutation or *ALK* fusion, a platinum-based doublet therapy is favored. Patients with actionable driver mutations who are initially treated with targeted medicine, eventually develop resistance and subsequent chemotherapy is indicated. Targeted therapy with concurrent chemotherapy might be beneficial and studies on this subject are ongoing. Prior research did not indicate increased survival when concurrent chemotherapy was combined with EGFR-inhibitors. (Lwin, et al., 2013)

Chemotherapeutic substances have distinct dose-limiting side effects, some of which can be treated with other medicines and others not. Chemotherapeutic agents can cause both central and peripheral neurotoxicity. Transient bone marrow suppression, leading to neutropenia, anemia, and thrombocytopenia, is common side-effect of most chemotherapeutic drugs. Neutropenia is a significant rate-limiting factor with many drugs, including docetaxel and paclitaxel. Cardiac toxicity leading to myocyte death and subsequent heart problems are common with anthracyclines. Nephrotoxicity can lead to hemolytic-uremic syndrome. Ocular toxicity ranging from mild conjunctivitis to permanent blindness are reported with a plethora of agents. Ototoxicity is commonly attributed to cisplatin and carboplatin. Other complications

include pulmonary reactions, mucositis, nausea and vomiting, constipation, gastrointestinal hemorrhage, pancreatitis, hepatotoxicity, hemorrhagic cystitis, and cutaneous reactions. (Livshits, et al., 2014)

Dose-limiting aspect of cisplatin is nephrotoxicity, other side-effects include cumulative peripheral sensory neuropathy, ototoxicity, nausea, and vomiting. Many tumors have intrinsic resistance to this drug, while other tumors gradually build tolerance as the treatment progresses. (Dilruba & Kalayda, 2016)

Carboplatin has a similar toxicity profile to cisplatin, but the overall toxicity is markedly lower. High-dose chemotherapy can therefore be performed with this agent. Myelosuppression is the dose-limiting side-effect of carboplatin, but modern hematopoietic colony-stimulating actors can be used to counter this. Dose-limiting side effect for third generation platinum-based drug oxaliplatin is neurotoxicity. (Dilruba & Kalayda, 2016)

2.4 Immunotherapy

Immune checkpoint inhibitors (ICIs) have drastically improved the treatment of late-stage NSCLC. These agents function by blocking inhibitory pathways that tumors can exploit to physiologically evade immune system. In the case of NSCLC, the ICIs of interest are PD-1, PD-L1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). The mechanisms of these molecules heavily revolve around T cells, which in turn modulate the natural immune response to foreign entities, including cancers, in the human body. (Proto, et al., 2019) Although targeted therapies are under intensive development, majority of the patients have no targetable alterations in their tumor genetical make up and it is these patients that have gained the benefits of immunotherapy. Immunotherapy increases OS and quality of life in advanced NSCLC and is routinely used. (Hanna, et al., 2020)

The tumor proportion score (TPS) describes the relative amount of tumor that expresses PD-L1, a transmembrane protein that suppresses immune response against the cell. Higher TPS predicts better response when anti-PD-1 and anti-PD-L1 therapies are used. The American Society of Clinical Oncology and Ontario Health NSCLC Expert Panel have made recommendations for treating stage IV NSCLC patients without driver mutations. The recommendations differ according to tumor histology and TPS. (Hanna, et al., 2020)

Patients with TPS ≥50% clearly benefit from immunotherapy. For patients with non-LUSC and TPS ≥50%, single agent pembrolizumab is recommended. (Hanna, et al., 2020) KEYNOTE-042 trial included 1,274 randomly assigned participants, 47% of which had a TPS ≥ 50%. Single agent pembrolizumab plus chemotherapy doublet (treatment arm) and chemotherapy doublet (control arm) were compared. When comparing all patients, the addition of pembrolizumab significantly increased OS (HR 0.69, 95% CI, 0.56-0.85; p=0.0003). Treatment arm saw 2-year OS improve from 30% to 50%. Adverse effect for patients with TPS ≥ 1% were less prominent in the treatment arm. KEYNOTE-042 trial did not find statistically significantly greater OS with treatment arm (HR, 0.82; 95% CI, 0.63-1.07; p=NS), however treatment arm was numerically favored. Favorable toxicity profile also makes the treatment less demanding for the patient. (Mok, et al., 2019)

KEYNOTE-189 compared pembrolizumab plus platinum doublet (treatment arm) with placebo plus platinum doublet (control arm) in patients with non-LUSC NSCLC. The treatment arm had greater OS (HR, 0.42; 95% CI, 0.26-0.68). While these results inevitably question the recommendations based on KEYNOTE-042 trial, the combination therapy is more toxic and financially demanding, which must affect decision making. (Mok, et al., 2019, Gadgeel, et al., 2020) Optional therapy with pembrolizumab plus platinum-doublet is a viable alternative for patients with non-LUSC TPS ≥50%. Trials comparing the effectiveness of pembrolizumab monotherapy versus pembrolizumab plus platinum doublet are lacking. (Hanna, et al., 2020)

For patients with LUSC and TPS ≥50%, single agent pembrolizumab is recommended (Hanna, et al., 2020). KEYNOTE-042 study concluded that patients with LUSC and TPS ≥ 50% benefit from single-agent pembrolizumab (HR 0.53, 95% CI, 0.38-0.75) (Mok, et al., 2019). KEYNOTE-407 study found the combination of pembrolizumab plus platinum doublet to have 1-year survival of 63.4% versus 51% for those receiving placebo plus platinum doublet HR for OS was 0.64 (95% CI, 0.37-1.10), these finding however, were not statistically relevant. (Paz-Ares, et al., 2018)

Patients with TPS negative (0%) or low positive (1%-49%) also seem to benefit from immunotherapy. For patients with non-LUSC and TPS negative or low positive, pembrolizumab plus platinum doublet (carboplatin/pemetrexed) is recommended. (Hanna, et al., 2020) KEYNOTE-189 showed clear survival benefit for pembrolizumab plus platinum doublet versus placebo

plus platinum doublet in patients with TPS 1% to 49%. Twelve-month survival rate was 71.5% in the treatment arm and 50.9% in the control arm (HR, 0.55; 95% CI, 0.34-0.90). Patients with TPS <1% also benefitted from treatment (HR, 0.59; 95% CI, 0.38-0.92). (Gadgeel, et al., 2020) Optional treatment for non-LUSC NSCLC with TPS <50% is the combination of atezolizumab plus bevacizumab plus platinum doublet. This combination is particularly promising for patients with *EGFR* mutation or *ALK* rearrangements, that have experienced disease progression after tyrosine kinase inhibitor (TKI) therapy. (Proto, et al., 2019)

For patients with LUSC and TPS negative (0%) or low positive (1%-49%), pembrolizumab plus platinum doublet (carboplatin/(nab-)paclitaxel) or chemotherapy alone is recommended. (Hanna, et al., 2020) The KEYNOTE-407 study compared pembrolizumab plus platinum doublet versus chemotherapy alone. The subgroup with TPS <1% had greater OS than control arm (HR 0.61, 95% CI 0.38-0.98) and as did the subgroup with TPS 1%-49% (HR 0.57, 95% CI 0.36-0.90). (Mok, et al., 2019)

Ipilimumab, a monoclonal antibody targeting the CTLA-4 protein is another novel option for the treatment of NSCLC. It offers a viable alternative, not on its own but rather in the form of combined immunotherapy. Ipilimumab combined with nivolumab (PD-1 antibody) was tested in a phase III trial against chemotherapy or several combined therapies. In patients with TPS>1%, ipilimumab plus nivolumab proved superior against chemotherapy as results showed median duration of OS of 17.1 months (95% CI, 15.0-20.1) versus 14.9 months (95% CI 12.7-16.7), 2-year survival of 40.0% versus 32.8%, and median duration of response of 23.2 months versus 6.2 months, respectively. Ipilimumab plus nivolumab against nivolumab monotherapy alone showed 2-year OS of 40% versus 36.2% and median duration of response of 23.2 months (95% CI 15.2-32.2) versus 15.5 months (95% CI 12.7-23), respectively. The effect of combination therapy versus nivolumab alone was even more prominent for patients with TPS>50%, as 2-year OS was 48.1% versus 41.9% and median duration of response was 31.8 months (95% CI, 18.7 to not reached) versus 17.5 months (95% CI 13.5-31.0), respectively. Patients with TPS<1% also saw benefit when ipilimumab plus nivolumab was used instead of chemotherapy. Median duration of OS was 17.2 months (95% CI, 12.8-22.0) versus 12.2 months (95% CI, 9.2-14.3), respectively. Ipilimumab plus nivolumab was also superior to nivolumab plus chemotherapy, as 2-year OS was 40.4% and 34.7%, while median duration of response was 18 months and 8.3 months, respectively. All patients accounted showed less severe reactions to combined immunotherapy (32.8%) versus chemotherapy (36.0%). (Hellmann, et al., 2019)

One meta-analysis compared the effectiveness of ipilimumab plus nivolumab against pembrolizumab monotherapy, pembrolizumab plus chemotherapy, nivolumab monotherapy and chemotherapy alone. In terms of effectiveness, the combination of ipilimumab plus nivolumab was superior to nivolumab monotherapy, pembrolizumab monotherapy and chemotherapy, but inferior to pembrolizumab plus chemotherapy. The combination of ipilimumab plus nivolumab however, was generally well tolerated compared to other existing immunotherapeutic treatments. (Ando, et al., 2020)

In stage III NSCLC, the concurrent use of durvalumab (PD-1 antibody) with radiotherapy increased OS as discussed earlier. 4-year follow-up of a phase-3 RCT (PACIFIC) showed the addition of durvalumab after concurrent chemoradiotherapy to significantly improve OS, when compared against placebo (HR, 0.68; 95% CI, 0.53-0.87, p=0.00251). (Faivre-Finn, et al., 2021)

Adverse effects related to immunotherapy can occur in any organ system and can occur within days of initiation to a full year, median onset however, is usually between 2 to 16 weeks. Drugs targeting CTLA-4 are more prone to cause adverse effects than PD-1 and PD-L1 inhibitors, but the mechanisms underlying this difference are not well known. (Ramos-Casals, et al., 2020)

Large meta-analysis of 36 phase II/III trials have estimated the general safety of immunotherapy. Pooled incidence of all adverse effects ranged between 54% to 76% (Xu, et al., 2018).

The prevalence of endocrinopathies is >10% for CTLA-4 inhibitors and 4-14% for anti-PD-1 inhibitors. Most common endocrinopathies are hypophysitis, thyroid dysfunction and type 1 diabetes mellitus. Gastrointestinal adverse effects are rather common. The incidence rates of diarrhea for PD-1 inhibitors, CTLA-4 inhibitors and combined therapy are 20%, 35% and >40%, respectively. Colitis has a prevalence of 12%, 1% and 14% respectively. The prevalence of hepatitis is 1-6%, 1-25% and 17-22%, respectively. Hematological adverse effects, most commonly neutropenia, autoimmune hemolytic anemia, immune thrombocytopenia, and aplastic anemia are rare (3-4% of total adverse effects). Severe dermatological adverse effects (grade 3 or more) occur in 2-10% of patients receiving immunotherapy. Less than 1% of patients experience cardiac toxicity. However, heart related adverse effects, such as myocarditis, can potentially be fatal. (Ramos-Casals, et al., 2020)

2.5 Targeted therapy

Oncogenic driver mutations are identified in 60% of LUADs and in 50% to 80% of LUSCs. Targeted therapies against identifiable driver mutations are now routinely used in advanced NSCLC. EGFR and ALK are the most common therapeutic targets, while other potential targets of interest include gene rearrangements of *ROS1* and *RET*-proto-oncogene, activating mutations in *BRAF*, *KRAS* and human epidermal growth factor receptor 2 genes and amplification of tyrosine-protein kinase Met gene (Zappa & Mousa, 2016, Schroff, et al., 2018). ECOG-1505 trial showed no benefit for use of targeted therapies against early-stage NSCLC, but this trial did not specifically direct the therapy against patients with targetable mutation (Dahlberg, et al., 2017). The ongoing ALCHEMIST screening trial is exploring the use of EGFR-inhibitors and ALK-inhibitors in their respective mutant populations (NCI, 2021).

EGFR is a cell surface receptor that under dimerization, activates tyrosine kinases, which in turn advances several tumorigenic processes, such as cell proliferation, angiogenesis, adhesion, motility, and apoptosis. *EGFR* mutations are more prevalent in adenocarcinomas, women, never-smokers, and Asians. (Schroff, et al., 2018) Approximately 15% of LUADs in the USA harbored *EGFR*-mutations. US Food and Drug Administration (FDA) has approved the use of four tyrosine kinase inhibitors gefitinib, erlotinib, afatinib and osimertinib for clinical use. Several studies have established the role of TKIs in advanced NSCLC with *EGFR*-mutant patients. (Naylor, et al., 2016)

Therapeutic potential of EGFR-inhibitors has been investigated in several trials (Table 4). First-generation gefitinib and erlotinib are reversible competitive adenosine triphosphate inhibitors and target only EGFR. A multicenter, open-label phase III RCT (OPTIMAL) showed significantly longer PFS of 13.1 months with erlotinib compared to platinum-doublet (Zhou, et al., 2011). Erlotinib demonstrated potential also in another phase III trial (ENSURE), when both PFS and objective response rate (ORR) were considerably better than with platinum doublet (Wu, et al., 2015). Another multicenter, open-label, phase III RCT (EURTAC) showed erlotinib to have significantly higher PFS (9.7 months) than standard chemotherapy (Rosell, et al., 2012). In one phase III RCT (WJTOG-3405), gefitinib also showed similar results compared to platinum-based chemotherapy (Yoshioka, et al., 2019). Second-generation TKIs, such as afatinib and dacomitinib function as irreversible inhibitors that target EGFR, receptor tyrosine-protein kinase erbB-2 and receptor tyrosine-protein kinase erbB-4. The LUX-LUNG-7 and ARCHER-

1050 trials demonstrated that afatinib and dacomitinib, respectively, have superior PFS compared to gefitinib. (Paz-Ares, et al., 2017, Wu, et al., 2017)

Disease progression inevitably occurs after the initial response, usually after 1-2 years. Up to 63% of patients treated with TKIs have shown to harbor *EGFR T790M* mutation upon disease progression. After the tumor develops resistance to first generation TKIs, afatinib and dacomitinib can be used. In this scenario, however, response rates less than 10% and PFS less than 4 months can be achieved. (Jänne, et al., 2015) Subsequently, osimertinib and rociletinib have been approved as second line therapy (Naylor, et al., 2016). For patients with T790M-positive NSCLC who progressed after initial TKI treatment, osimertinib showed ORR of 61% and median PFS of 9.6 months (Jänne, et al., 2015). Platinum-based chemotherapy is used for patients with T790M-negative NSCLC who progress after initial TKI therapy (Hirsch, et al., 2017). Third generation TKIs have also been tested against other TKIs in untreated *EGFR*-positive NSCLC population. Indeed, both Osimertinib (FLAURA) and dacomitinib (ARCHER-1050) showed better ORR and PFS than conventional TKIs. (Wu, et al., 2017, Soria, et al., 2018)

Table 4: Selected randomized trials with first line TKI therapies

Study	Design	ORR (%)	PFS (months)
IPASS	Gefitinib vs carboplatin plus paclitaxel	72.1 vs 47.3	9.5 vs 6.3
NEJ002	Gefitinb vs carboplatin plus paclitaxel	73.7 vs 30.7	10.8 vs 5.4
WJTOG-3405	Gefitinib vs cisplatin plus docetaxel	62.1 vs 32.2	9.2 vs 6.3
EURTAC	Erlotinib vs platinum doublet	58 vs 15	9.7 vs 5.2
ENSURE	Erlotinib vs cisplatin/gemcitabine	63 vs 34	11.0 vs 5.5
OPTIMAL	Erlotinib vs carboplatin plus gemcitabine	83 vs 36	13.1 vs 4.6
LUX-Lung-3	Afatinib vs cisplatin/pemetrexed	56 vs 23	11.1 vs 6.9
LUX-Lung-6	Afatinib vs cisplatin/gemcitabine	67 vs 23	11.0 vs 5.6
LUX-Lung-7	Afatinib vs gefitinib	72.5 vs 56	11 vs 10.9
Archer-1050	Dacomitinib vs gefitinib	75 vs 72	10.9 vs 7
FLAURA	Osimertinib vs gefitinib or erlotinib	80 vs 76	18.9 vs 10.2

ALK rearrangements are found in 5% of unselected NSCLC. The prevalence is up to 22% in never- or light smokers. Adenocarcinomas harbor nearly 97% of all *ALK* rearrangements. Normal ALK is functional in growing tissue and suppressed when tissue reaches maturity. It functions by activating several pathways, including Janus kinase/signal transducers and activators of transcription, and mitogen-activated protein kinase (MAPK). The echinoderm microtubule-associated protein-like 4 is the most common ALK fusion protein partner. ALK inhibitors have been established as the primary therapy against *ALK*-positive advanced NSCLC. (Friedlaender, et al., 2019) In the PROFILE 1014 -trial, first generation TKI crizotinib showed superior results when compared to chemotherapy. Results showed ORR 74% versus 45%, PFS 10.9 months versus 7.0 months, 1-year survival of 84% versus 79% and 4-year survival of 56.6% versus 49.1%, respectively. (Solomon, et al., 2018)

As with other targeted therapies, disease progression is common. 31% of patients with *ALK*-positive NSCLC treated with crizotinib have experienced disease progression at 18 months. First generation ALK-TKIs permeate blood brain barrier poorly, and subsequently, 50% of pa-

tients treated with crizotinib have central nervous system progression during the treatment. (Friedlaender, et al., 2019) To counter these issues, second generation ALK-TKIs were developed. Indeed, one phase III RCT (ASCEND-5) demonstrated the use of ceritinib instead of platinum-based chemotherapy as second line treatment after disease progression to improve PFS (5.4 versus 1.6 months, HR 0.49, 95% CI 0.36-0.67; p<0.0001)). (Shaw, et al., 2017)

Second generation ALK-TKIs have since taken their place as first line treatment in *ALK*-positive NSCLC. Alectinib was compared to crizotinib as first line treatment in phase III RCT (ALEX). Updated results showed median PFS to be 34.8 months for alectinib and 10.9 months for crizotinib. (Camidge, et al., 2019)

Should disease progression occur after treatment with afatinib, third generation TKI lorlatinib is recommended, as it remains effective against several common resistance mechanisms. When lorlatinib was used to treat *ALK*-positive NSCLC patients in a treatment naïve cohort, second line after crizotinib cohort, second line after second generation TKIs cohort and in patients who had received up to three TKIs prior, the ORRs were 90%, 69.5%, 32.1% and 38.7%, respectively. (Friedlaender, et al., 2019)

ROS1 rearrangements are present in 1-2% of NSCLC. Several variants have been discovered and they are most prevalent in younger-, female- and light- or never-smoking patients. Crizotinib received approval from the FDA when positive response was recorded in *ROS1*-positive patients mostly pretreated with chemotherapy (72% of patients responded, median PFS 19.2 months). (Hirsch, et al., 2017)

Mutations in *BRAF* are another potential driver in NSCLC tumorigenesis that function by stimulating the MAPK pathway. Several genetic alterations are known, with V600E being the most prevalent. *BRAF* alterations are observed in 3-5% of lung cancers, mostly in smokers with LUAD. (Hirsch, et al., 2017) Functional studies have shown that concomitant blocking of both BRAF and mitogen-activated protein kinase kinase (MEK) leads to significantly increased tumor regression and therefore, both FDA and European Medicines Agency have approved the use of BRAF inhibitor dabrafenib plus MEK-inhibitor trametinib for the treatment of *BRAF V600E* mutated metastatic NSCLC regardless of previous therapies. (Leonetti, et al., 2018)

Angiogenesis is a core component in several cancers, including NSCLC. Angiogenetic inhibitor bevacizumab in combination with chemotherapy is approved in patients with metastatic non-LUSC NSCLC. (Manzo, et al., 2017) Bevacizumab plus platinum doublet was compared against platinum doublet alone in first line setting for advanced NSCLC (E4599). The addition of bevacizumab increased both OS (12.3 versus 10.3 months, HR 0.79, p=0.003) and PFS (6.2 versus 4.5 months, HR 0.66, p<0.001). (Sandler, et al., 2006) Novel antiangiogenic agents ramucirumab and nintedanib have also shown promising results. Ramucirumab plus docetaxel was compared to docetaxel alone (REVEL). Results showed significant improvement in median OS (10.5 months versus 9.1 months, HR 0.86, p=0.023) and PFS (4.5 versus 3.0, HR 0.76, p<0.0001). (Garon, et al., 2014) Two phase III RCTs compared nintedanib plus docetaxel to docetaxel alone in second line setting in patients with advanced NSCLC. LUME-LUNG 1 showed that for patients with any histology, nintedanib plus docetaxel produced increased PFS than docetaxel alone (3.4 versus 2.7 months, HR 0.79, p=0.0019), OS was not significantly different (Reck, et al., 2014). LUME-LUNG-2 showed nintedanib plus docetaxel to increase PFS in non-squamous histology NSCLC (4.4 versus 3.6 months, HR 0.83, p=0.04), OS was not statistically different. (Hanna, et al., 2016). Ramucirumab/nintedanib plus docetaxel is therefore an option for the second line treatment of patients with advanced LUAD NSCLC. (Manzo, et al., 2017)

Wide range of adverse effects are associated with targeted therapies (Table 5). However, their toxicity profile is generally safer than cytotoxic chemotherapy. (Naylor, et al., 2016, Manzo, et al., 2017)

Table 5: Possible adverse effects in selected targeted therapies

Class	Drugs	Adverse effects
EGFR	Erlotinib, afatinib, gefitinib, Osimertinib, rociletinib	Rash, diarrhea, anorexia, fatigue, dyspnea, cough, nausea, vomiting, interstitial lung disease, hepatotoxicity
ALK	Alectinib, ceritinib, brigatinib, crizotinib	Vision disorder, diarrhea, edema, hepatotoxicity, vomiting, constipation, dysgeusia, fatigue, pyrexia, pneumonitis
BRAF	Vemurafenib, dabrafenib	Other malignancies, hypersensitivity reactions, dermatologic reaction, QT prolongation, hepatotoxicity, uveitis
VEGF	Bevacizumab, nintedanib, ramucirumab	Diarrhea, hepatotoxicity, nausea, hypertension, proteinuria, bleeding, neutropenia, leucopenia, fatigue, vomiting.

3 Bacteria-based therapies

Bacteria-based treatments are an interesting topic of cancer research. Bacteria possess several mechanisms that make them unique in this field. Their ability to penetrate tissue, home in on tumors, controlled cytotoxicity, ability to transform therapeutic molecules and easy detectability are attributes that are unachievable with standard therapies. *Salmonella enterica Serovar Typhimurium* along with some other facultative and obligate anaerobes have shown to accumulate in various tumors in mice, subsequently resulting in the inhibition of tumor growth. This phenomenon is believed to occur because of immune system activation and/or competition for nutrients. (Forbes, 2010)

Bacteria's intrinsic cytotoxicity combined with their ability to actively secrete virulence proteins and cytotoxic cargo directly into the cell allows for dual approach (Nishikawa, et al., 2006).

Several challenges remain, for often increased tumor-homing and the ability to colonize tumors comes with increased virulence, which could be problematic for immunocompromised late-stage disease patients. Therefore, controlling bacterial toxicity will be a priority before regula-

tory approvals can be achieved. (Forbes, 2010)

Although trials for bacteria-based therapies are beginning to emerge, available treatments have remained scarce. Moxetumomab pasudotox is a recombinant immunotoxin. In this drug, a fragment of anti-CD22 monoclonal antibody is fused to a fragment of a Pseudomonas exotoxin. The FDA has approved the use of this drug for patients with relapsing or refractory hairy cell leukemia. (Falini & Tiacci, 2019)

One phase I/II trial utilizes immunotherapy based on attenuated *Listeria monocytogenes* bioengineered to elicit immune response against antigens commonly found in NSCLC. The strain (A503) is used in combination with pembrolizumab as their mechanisms complement each other. Toxicity profile of A503 was deemed safe for patients with metastatic LUSC or non-LUSC NSCLC. A503 alone induced immune response and further results are expected in 2023. (Ramalingam, et al., 2020)

Another phase I trial tested *Listeria monocytogenes*-based vaccines and their ability to stimulate both innate and adaptive immunity. Results showed generally safe toxicity profile, indicating the possibility for further trials. Patients with mesothelioma, lung, pancreatic and ovarian cancers as well as patients with liver metastases were evaluated. Immune activation, measured by serum cytokine levels and natural-killer cell activation, was observed. (Le, et al., 2012)

4 Discussion

For the most part, cancer remains a disease of the elderly. Modern therapeutic successes on cardiovascular diseases, infections and other historically demanding diseases have left the role of cancer be ever more central in the future. The dual effect of decreasing smoking and ageing population is poised to increase the relative percentage of non-LUSC NSCLC, thus providing a fruitful ground to develop novel treatments, as mutational burden and 5-year prognosis are generally better. Early diagnosis still proves to be challenging, as prognosis is directly bound to cancer stage at the time of diagnosis. but the inevitable shift towards more advanced therapies will keep progressing, as trials are performed to expand the use of targeted therapies and immunotherapies into earlier stages. Genetic profiling of the tumor will remain the key aspect in their use. Surgery, radiotherapy, and chemotherapy will remain the backbone of lung cancer treatment for some time and they also are developed simultaneously.

Resurfaced interest in bacteria-based therapies will be an interesting approach, as the natural capabilities of bacteria and their limitless potential for enhancements can be widely exploited to serve therapeutic effort through multiple mechanisms, either as a monotherapy or by complimenting another treatment (Sedighi, et al., 2019).

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Appendices
Appendix 1 – Abbreviations

ALK	Anaplastic lymphoma kinase
BRAF	v-Raf murine sarcoma viral oncogene homo-log B
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
EGFR	Epidermal growth factor receptor
EP	Etoposide/cisplatin
ERBT	External beam radiotherapy
FDA	US Food and Drug Administration
FDG	Fluorodeoxyglucose
FGFR	Fibroblast growth factor receptor
Gy	Gray
HR	Hazard ratio
ICI	Immune checkpoint inhibitor
KRAS	Kirsten Rat Sarcoma
LACE	Lung Adjuvant Cisplatin Evaluation
LUAD	Lung adenocarcinoma
LUSC	Lung squamous-cell carcinoma
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
NOS	Not otherwise specified
NSCLC	Non-small-cell lung carcinoma
ORR	Objective response rate
OS	Overall survival
PC	Carboplatin/paclitaxel
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PET	Positron emission tomography
PFS	Progression-free survival
PM2.5	Fine particulate matter
RCT	Randomized controlled trial
ROS1	Proto-oncogene tyrosine-protein kinase ROS
RR	Risk ratio
SBRT	Stereotactic body radiation therapy
SCLC	Small-cell lung cancer
SEER	Surveillance, Epidemiology, and End Results Program
TKI	Tyrosine kinase inhibitor
TNM	TNM Classification of Malignant Tumors
TP53	Tumor protein P53
TPS	Tumor proportion score
VATS	Video-assisted thoracoscopic surgery
VEGF	Vascular endothelial growth factor