

# INSIGHT INTO THE MOLECULAR TARGETS AND DRUG DEVELOPMENT AGAINST LUNG CANCER

## Abstract

With an estimated 2 million diagnoses and 1.8 million deaths worldwide, lung cancer is the primary cause of cancer incidence and mortality. Lung neoplasms represent the second most prevalent cancer diagnosis for both genders. However, there are significant regional variations in lung cancer incidence and mortality rates, which can be attributed to genetics, exposure to environmental risk factors, and different tobacco smoking behaviors. Lung cancer is expected to be the most common cancer worldwide by 2050. Lung cancer tops the list in part because it is frequently discovered only when the disease has progressed to an advanced stage. Thus, early detection of lung cancer is critical, particularly when screening high-risk groups like smokers, those who have been exposed to pollutants, those who work in oil fields, those who work in hazardous environments, etc. Different forms of imaging are used in the diagnosis of lung cancer nowadays, along with pathological evaluation of samples; nevertheless, these methods are still unable to identify early stages of the disease with accuracy. Effective early detection, a thorough etiology, and the right medications all contribute to lung cancer treatment. Early detection of lung cancer is critical, particularly when screening high-risk individuals and searching for new biomarkers urgently. Furthermore, the best course of action for each patient with lung cancer depends on a precise diagnosis. In this chapter we discuss the screening techniques, pathways and targets that are exploited for the treatment of lung cancer.

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## I. INTRODUCTION

Lung cancer is the leading cause of death among men and women, which accounts for 350 deaths per day United States in 2022[1]. It is generally subdivided into a small cell (SCLC) and non-small cell lung cancer (NSCLC) types. The absence of sensitive tests for early diagnosis of lung cancer and ineffective treatment regimens for locally and advanced metastatic disease is the root cause of increased lung cancer prevalence[2, 3]. With the broad endeavors for tobacco awareness education, development of imaging, and consolidated treatment modalities, it was observed a 5 year endurance pace of lung cancer improved by 12% (in 1977) to 16% (in 2007) [1]. Although lung cancer is diagnosed at an early stage, then complete resection might help improve 5-year survival by 67%[4]. Thus, it is accomplished that early diagnosis of lung cancer disease by sensitive screening test may be used as a crucial strategy to improve the prognosis of affected lung cancer patients and reduce mortality incidence[5].

Smoking causes more than 80% of cancers in the Western world, and advances in smoking cessation have reduced morbidity and mortality. Continuing to smoke, with other risks such as occupational exposure to asbestos and combustible gases, as well as environmental exposure to arsenic and air pollution, remains important in countries where it is created. Cancer is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) based on cell of origin, and these are further divided. According to the 2015 World Health Organization classification, the most common types of lung cancer include adenocarcinoma (adenocarcinoma), squamous cell carcinoma (SCC) and cell carcinoma (SCLC), neuroendocrine carcinomas such as large cell neuroendocrine carcinoma (LCNEC), and carcinoid [6]. Carcinoid tumors are tumors of well-differentiated cells of neuroendocrine cells (Kurczycki cells), whereas small carcinoid tumors also originate from poorly differentiated cells and cause rapid metastasis, poor response to treatment, and poor prognosis. Squamous and small cell carcinomas are more likely to be associated with moderate and smoking history, especially for men. Adenocarcinomas are more common in women and nonsmokers, adenocarcinomas are of peripheral origin, and the discovery of driver mutations such as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), BRAF and ROS1 is positive. Small molecule inhibitors of receptor tyrosine kinases target these changes in combination with anti-inflammatory agents such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors. recent years. Modify or add chemotherapy for eligible patients [7].

- 1. Screening of Lung Cancer:** Several useful screening tools are exploited for early detection of lung cancer patients, including chest X-ray (CXR) or computed tomography (CT) employed with or without sputum sampling, LDCT, circulating DNA and RNA, serum biomarkers, CTC, exosomal microRNA will be reviewed further.
- 2. CXR:** In the early 1980s, numerous randomized control trials have been performed using plain CXR and sputum cytology at Mayo clinic. In the randomized trial of high-risk patients, 9211 contributors were selected from 10,933, aged over 45 to CXR and sputum cytology assigned as the control group versus repeated CXR and sputum cytology analysis for a span of 6 years. Studies suggest 206 cases were diagnosed with lung cancer, and 160 cases were in the control group with significantly improved screening for early diagnosis and 5- year survival of lung cancer patients. Although statistically, studies do

not demonstrate disease-specific mortality difference among the two studied groups from lung cancer, this remains in the case with the follow-up extended to over 20 years[8–12].

The MSKLP and JHLP is a randomized trial of participants aged more than 40 years was done annually where analysis of CXR in the presence (screening group) or absence (control group) of sputum cytology was checked every four months. In the MSKLP study, 10,040 participants were enrolled, and 144 cases were diagnosed in both groups, but no difference was observed in overall survival, stage distribution, and disease-specific mortality amongst the two groups [8, 9] [13, 14]. In the JHLP study, 10,382 participants and around 194 cases with affected lung cancer were reported in the screening group, whereas 202 were in the control group. Similar to the MSKLP trial, the JHLP study did not show any difference in overall survival or disease-specific mortality amongst the two groups [15–17]. Two studies were done at Johns Hopkins and Memorial Sloan-Kettering cancer centers that involved 10,000 participants each, compared plain CXR in the presence and absence of sputum cytology. In patients who developed lung cancer accomplices with dual screening, nearly 20% were diagnosed by cytology alone (most probably early-stage squamous cell carcinomas). However, there seems to no difference in mortality by adding cytology screening [14, 17].

3. **Low Dose CT Screening:** CT is more effective than CXR as it offers a more detailed image of the chest and is more helpful in diagnosing cancer. Although, it is mostly accepted that the radiation dose of LDCT, which is approximately 1000 times greater than CXR, is too high to assist the early diagnosis of lung cancer to exceed radiation exposure danger. Hence, until CT was approved at lower radiation doses, there was a reestablished appetite for lung cancer screening. LDCT generally has 22% of effective radiation dose when compared to standard CT. LDCT screening reflects the risk of radiation prompting cancer, which was recently estimated by a Milan study that screened 4 per 10,000 patients with a radiation dose of follow up PET CTs for patients with a positive LDCT scan (carrying high radiation doses). Adjusting this risk against the advantages of screening, the authors related to this study suggested that LDCT can be viewed as safe. However, alternative protocols have been suggested to reduce the usage of PET CTs in the screening tool to mitigate the risks of radiation exposure.
4. **Selecting the Target Population:** Screening of lung cancer needs to target those who are likely to at high risk of lung cancer. As such, screening of never smokers was found to be ineffective.
5. **Bronchoscopy:** Bronchoscopy is the widely used diagnostic tool, firstly performed by Gustav Killian of Freiburg, Germany, in 1887[18], employing endobronchial ultrasound (nodal staging of the lung cancer) [19, 20]. Bronchoscopy is commonly used for indicating tissue sampling and determining the degree of lung cancer [21]. Several diagnostic accessories can be introduced by the working channel of the flexible bronchoscope. These accessories include brushes, biopsy forceps, needles, and an immense role in diagnosing and staging lung cancers. Their combined effect has significantly improved in obtaining pulmonary biopsies, specifically of ever-smaller lesions. Computed tomography (CT) has emerged as the current cornerstones of imaging techniques [22]. Autofluorescence bronchoscopy (AFB) profited by perceiving that the

emission spectrum of the bronchial mucosa under blue light fluctuates when dysplastic or carcinomatous lesions develop[23, 24].

- 6. Liquid Biopsies:** Liquid biopsies or blood-borne biomarkers is gaining much attention for monitoring the advanced stage lung cancers. Liquid biopsies include circulating proteins, circulating nucleic acid, or circulating tumor cells (CTCs). The limitation lies in its sensitivity and specificity for the early diagnosis of lung cancer[25].
- 7. Circulating Mirnas in Lung Cancer Diagnosis:** MicroRNAs (miRNAs) are important regulators of gene expression, acting through transcriptional repression or degradation of mRNA targets. Changes in miRNA expression have been implicated in the pathogenesis of many cancers [26]. An example of this includes let7 miRNA, which is downregulated in most lung cancer tissues and upregulated in suppressed lung cancer cell lines [27]. Studies have shown that exosomes produced by cancer cells [28] increase the long-term guidance time and prepare them for metastatic disease, which is a good scientific discipline. The power of miRNA profiling has been fully exploited to improve the performance of lung cancer diagnosis. Boeri *et al.* [29] tested miRNA expression in plasma of patients in LDCT lung examination to differentiate miRNAs before lung cancer development and prognosis [30] for identifying differentially expressed miRNAs before the development and diagnosis of lung cancer. The inclusion of miRNAs in early diagnosis appears to be a promising NSCLC diagnostic tool, but it is now important to establish a well-established, independent tool and there is good research to prove it is worth using.
- 8. Antibodies in Lung Cancer Detection:** It is well-known that the hereditary distortion included within the handle of carcinogenesis leads to distinctive expressions of ‘self-antigens’ either by unseemly expression of tissue-specific proteins (neo-antigens) the items of non-synonymous quality mutations[31]. These tumor antigens are found to be at the interface among the resistant framework and creating cancers, [32], thus offers the likelihood of abuse as an early discovery biomarkers. The affiliation between the resistant framework and cancer is by and large complex, and the writing centers on the parts of cytotoxic T cells [33]. In any case, it has long been anticipated that the humoral safe framework may be dysregulated, coming about in autoantibodies that can be related with biomarker revelation [34]. Several investigations uncover the affiliation of antibodies with the occurrence of lung cancer. The primary was p53 antibodies, which exist in around 12% of lung cancer patients [35]. Certainly, the capacity that those may need to hold ended up underscored by utilizing the rise of p53 antibodies some time recently radiologically self evident lung cancers [36] related with lung cancer, which may constrain its utility in huge screening programs.
- 9. CTDNA in Lung Cancer Detection:** DNA is thought to enter the plasma passively through cell death (apoptosis or necrosis) or its release from living cells. In cancer patients, some cell-free DNA originates from the tumor and produces fragmented tumor DNA (ctDNA) [36]. The efficacy of ctDNA in lung cancer has been confirmed in NSCLC studies, where mutations have been identified and a library has been created to identify mutations associated with NSCLC. In the validation cohorts of healthy controls and NSCLC patients, sensitivity and specificity reached approximately 85% and 96%,

respectively. ctDNA is detectable in all advanced NSCLC cases, but only in 50% of early cases [37]. Total ctDNA was confirmed by sequencing the human telomerase (hTERT) gene. Connectivity levels were higher in NSCLC patients compared to gender/age/smoking matched controls using this method [38]. Recent advances in ctDNA therapy in personalized ctDNA assays based on biopsy-derived genomic landscapes to monitor patient response and hopefully prevent treatment and tumor development [39]. Mutations such as p53 can be used in lung cancer; but they are also seen in non-cancer smokers. [40]. In addition, new evidence is emerging of genetic mosaicism in healthy tissues, including mutations in genes that play an important role in cancer [41]. While candidate gene analysis using droplet digital PCR-based techniques is better understood, overall genetic variation will provide more insight into the presence of tumor cancer due to the need for next-generation sequencing.

**10. Circulating Tumor Cells in Lung Cancer Detection:** As cancer grows and progresses, cell subpopulations change their phenotype and become motile, invade surrounding tissue and invade blood vessels through multiple layers such as epithelial-mesenchymal transition [42], angiogenic mimicry [43], and cell cooperation [44]. These so-called CTCs are often heterogeneous and are assumed to have many cells responsible for distant metastasis [45]. In the field of cancer, this hypothesis is confirmed by the fact that CTCs produced from SCLC patients are tumorigenic in mice and produce responsive transplants. Treatment has been observed in primary patients [46]. There are many strategies for detecting CTCs [47], and in summary they have an important role in making quantitative and positive biomarkers of cancer. With the help of various CTC detection techniques, it seems that early detection can benefit. Extraction of tumor cells by size (ISET) detected CTCs prior to treatment in approximately 50% of NSCLC patients, compared with 39% in cell line studies. The combination of the two methods resulted in an improvement in 69% of patients [48]. Another study used a ligand-PCR method to quantify CTCs. After immunodepletion of leukocytes and erythrocytes, cells were labeled with oligonucleotide conjugated folate receptor ligand (FOLR1), allowing quantification by real-time PCR. This method was able to detect CTCs in 8 out of 10 stage I/II NSCLC patients, with an overall sensitivity of approximately 82% for the diagnosis of stage I-IV NSCLC patients [49]. A problem with the use of CTC analysis is the low frequency of CTC in advanced patients compared to the large number of blood cells in the sample. CTC heterogeneity has confounding marker-dependent capture, and not all CTCs are larger than blood cells, causing confusion based on size-based methods. In addition, any CTC enrichment step suffers from cell loss. Newer techniques, including high-throughput single-cell analysis platforms, are better for early detection because all cells in the sample can be easily analyzed using variable markers exchange, and cells can be physically viewed and stored for individual analysis [50].

**11. Sputum Analysis:** Preliminary findings of lung cancer diagnosis by sputum cytology are not satisfactory. However, there has been interest in studying mucus with cell counting and new molecular techniques. An example of this is the UK multicenter Lung SEARCH study, in which COPD patients were randomly assigned or not assigned to annual sputum cytology/cell count. Patients with positive cytology/cell count included chest CT and AFB [51]. MicroRNAs in sputum were also measured for early detection. A study in squamous cell carcinoma of the lung showed that a panel of three miRNAs (eg, mir-205,

mir-210, and mir-708) had a diagnostic sensitivity and specificity of approximately 72% in differentiating squamous patients, respectively. cell carcinoma and 95% from controls. There is also interest in linking DNA mutations with sputum samples for early detection of cancer [52]. Interestingly, a retrospective study correlating sputum samples prior to histological diagnosis of lung adenocarcinoma found that approximately 5 of 11 patients with KRAS-positive tumors had sputum KRAS changes between 1 month and 4 years prior to clinical examination [53].

**12. Exhaled Breath Analysis:** As a non-invasive and easily accessible model for the patient, the exhaled breath holds promise for emergency diagnosis. In respiratory medicine, NICE currently recommends exhaled nitric oxide for the diagnosis of asthma [54]. There are also some interesting studies using exhaled breath to diagnose lung cancer. Perhaps the most interesting is training dogs to distinguish between breast and lung cancer patients by checking for the presence of volatile organic compounds (VOCs) in breath samples collected for cotton wool soaked with silicone oil coated polypropylene. In a double-blind validation cohort, the specificity and sensitivity were both 99% [55]. However, a recent study with a similar design and sample size had a sensitivity of approximately 71% and a specificity of approximately 93% for the diagnosis of the lung cancer condition [56]. Ion mobility spectrometry provides a highly sensitive method for detecting volatile compounds in exhaled breath. Study of cancer patients was easily distinguished from the control group - Cyranose 320 contains a black carbon polymer that changes resistance to VOC adsorption. Comparison of health versus cancer patients produced "small" cancer in education with sensitivity and specificity of 71% and 92%, respectively, in an independent validation cohort [57].

## II. TREATMENT OF LUNG CANCER

1. Research into the molecular and cellular biology of cancer has uncovered a picture of the pathways and molecules that gradually lead to the development of cells into an entire lung cancer. These studies involve the identification of genetic and epigenetic changes in specific molecules that lead to activation of signaling pathways important in carcinogenesis. Some of these changes include so-called oncogenes and pain suppressor genes. In the search for therapeutic targets, special attention is required to identify single or multiple genes required for both the malignant phenotype and the survival of cancer cells. These are generally considered as oncogene addictions [58]. In lung cancer, commonly activated oncogenes may include MYC, KRAS, MET, CCND1, EGFR/HER1/ERBB1, HER2/ERBB2, EML4-ALK fusion, CDK4, and BCL-2 [59]. These targeted treatments yield longer progression-free survival, high response rates, and prolonged overall survival than the traditional cytotoxic chemotherapies[60–62].
2. **EGFR Pathway Inhibitors:** Some clinicopathological features were associated with frequency and gene amplification of EGFR mutations, including adenocarcinoma histology, female, non-smoker history, and East Asian people. This signature has been shown to have a probability of more than 50% mutation in the EGFR TK domain[63]. While a proportion of NSCLC patients with EGFR mutations may not respond to TKIs, the 'second' TK mutation (ie, T790M) is associated with resistance [64, 65]. Although EGFR mutant patients appear to significantly respond to EGFR TKI, protein

overexpression and EGFR amplification are associated with survival after EGFR TKI treatment because Akt is required for this to occur [66, 67]. Both erlotinib and gefitinib have been tested in randomized studies in combination with cytotoxic chemotherapy as first-line therapy for metastatic NSCLC. These studies did not find a survival benefit from adding the agent to treatment, although a retrospective analysis concluded that patients who do not smoke may benefit from chemotherapy [68, 69]. Cetuximab (humanized monoclonal antibody) binds to the extracellular domain of EGFR and has been examined in NSCLC. In addition, cetuximab is investigated in combination with chemoradiotherapy for NSCLC [70] and in combination with chemotherapy in neoadjuvant therapy for non-small resectable IB-IIIa level lung cancer [71]. In addition, other drugs targeting the EGFR pathway in clinical trials include lapatinib (for EGFR and HER2), panitumumab (for EGFR) and HK-272 (for EGFR) and for HER 2) [72].

- 3. Angiogenesis Inhibitors:** Angiogenesis (growth of new blood vessels from existing blood vessels) is essential for tumor growth to provide adequate oxygen and nutrients for tissue proliferation for targeted angiogenesis for cancer therapy [73, 74]. VEGF (vascular endothelial growth factor) is a growth factor that primarily follows angiogenesis in normal and neoplastic cells [75]. The VEGF family consists of approximately six growth factors (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor) and three receptors (VEGFR-1), VEGFR-2 and VEGFR-3 [72]. The VEGF/VEGFR pathway has often been found to be de-regulated in cancer [76], and VEGF overexpression is associated with proliferation and poor prognosis in NSCLC [77–79]. Several drugs have been developed and some are currently being investigated to target the VEGF/VEGFR signaling pathway [77–79]. The monoclonal antibodies targeting VEGF and the VEGFR TKIs are amongst the best studied [72]. Bevacizumab (Avastin), a monoclonal antibody [80, 81] generally binds to all subtypes of VEGF-A and has been studied in clinical trials. A recent study has shown that the addition of bevacizumab to paclitaxel and carboplatin confers a significant survival benefit in the first-line treatment of patients with painless NSCLC [81], thus, bevacizumab has recently been approved for use in NSCLC management. VEGFR TKI are small molecules that preferentially bind to the ATP pocket of the VEGFR intracellular domain of tyrosine kinases (TKs), thereby inhibiting the downstream pathway. These compounds are usually associated with other receptor TKs such as EGFR and c-KIT. One of the inhibitors developed, ZD6474 (Zactima), is an oral dual kinase inhibitor responsible for targeting VEGFR-2 and EGFR to treat lung cancer. Combining ZD6474 with docetaxel as second-line therapy for advanced NSCLC patients [82] enhanced progression-free survival as compared to docetaxel alone in a randomized phase II clinical study [83], and a confirmatory phase III trial has been initiated [72].

### III. PI3K/AKT/PTEN PATHWAY INHIBITORS

PI3Ks are important regulators of many cellular processes, including cell growth, cell proliferation, apoptosis and cytoskeleton rearrangement. In many cancer patients, the PI3K pathway is actively activated by a series of events including activation of the upstream receptor TKs (such as PDGFR and EGFR) or mutations occurring in PIK3CA, which encodes the catalytic subunit of PI3K [84]. Akt is the essential downstream effector of PI3Ks and is stimulated in NSCLCs [85]. While PIK3CA mutations are observed to occur

in about 4% of NSCLC tumors the expression of PTEN protein, which tends to inhibit the PI3K/Akt pathway, is often reduced or lost in lung cancers, signifying an alternate mechanism of activating this pathway [86, 87]. Preclinical trials of LY294002 (PI3K inhibitor) have shown that the agent augments the sensitivity of NSCLC cells to radiation and chemotherapy and phase I study of this agent is underway [85]. Many inhibitors have been developed against the mammalian target (mTOR) of rapamycin, the downstream target of PI3K signaling pathway. These inhibitors include rapamycin and its analogues, temsirolimus (CCI-779), AP23573 and everolimus (RAD001) [88]. These agents have shown promising anti-tumour activity in early clinical studies [72].

#### IV. RAS/RAF/MEK/ERK PATHWAY INHIBITORS

The RAS family of proto-oncogenes, HRAS, KRAS and NRAS, are plasma membrane-associated G proteins and are key regulators of signaling involved in the differentiation and survival growth and proliferation of normal cells [89]. The RAS/RAF/MEK pathway is activated in lung cancer by activating KRAS mutations (as at codon 12) that occur in approximately 20% of lung cancers, primarily adenocarcinomas [90]. Although the specific functions of HRAS, NRAS and KRAS have not been determined, KRAS mutations are responsible for approximately 90% of RAS mutations in cancer. KRAS mutations have been found in cancers caused by smokers and associated with poor survival [91]. In addition, KRAS and EGFR mutations appear to be synergistic in lung cancer [92], and KRAS mutations are associated with primary resistance to EGFR TKI therapy [93]. A number of drugs have been developed that express different components of the RAS pathway and are currently in clinical trials [89]. One of these, the farnesyl transferase inhibitors (FTIs), is one of the most studied drugs, while the two orally bioavailable FTIs, tipifarnib and lonafarib, have been compared in "studies in combination with cytotoxic therapy for lung cancer [94].

- 1. Tumor Suppressor Gene Therapy:** The p53 tumor suppressor is one of the important gatekeeper that becomes activated by multiple factors particularly oncogenes, DNA, hypoxia, damage, resulting in the expression of downstream genes that participate in cell-cycle arrest, aiding in DNA repair mechanism or apoptosis initiation. The p53 is commonly inactivated through mutation in lung cancer of around 90% of SCLC and 50% of NSCLC cases [95, 96]. Reactivation of p53 function by p53 mutants or loss of p53 in cancer cells leads to apoptosis of tumor cells [97], and therefore these findings have led to the improvement of pharmacological methods of reactivating p53. Studies have shown that gene therapy from gene replacement studies of p53 gene therapy using retroviral p53 expression vectors is safe and feasible, but vaccine evidence is weak, especially in patients with non-small cell lung cancer. [98]. FUS1 is a newly discovered cancer gene located on chromosome 3p21, and is a region that is usually deleted in lung cancer. Loss or absence of post mutation of FUS1 protein and exogenous overexpression of FUS1 in most SCLC and NSCLC has been found in most SCLC and NSCLC protein causes inhibition of tumor cell proliferation and apoptosis [99, 100].
- 2. Histone Deacetylase Inhibition:** Hypermethylation of the promoter region of the tumor suppressor gene demonstrates the epigenetic effect of gene silencing that plays an important role in tumor initiation and development [101] and therefore represents the



preferred target. Histone deacetylases (HDACs) facilitate modification of histones by limiting access to DNA by transcription factors and suppress gene transcription related to cell proliferation. HDAC inhibitors can restore silent genes and induce antiproliferative activity by controlling the expression in tumor cells. Many HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA), depsipeptide and valproic acid are undergoing clinical trials for lung cancer treatment [72].

- 3. Proteasome inhibitors:** The ubiquitin-proteasome system has significant role in protein homeostasis as it is involved in controlling the cell cycle, DNA transcription and degradation of proteins associated with healing, angiogenesis and apoptosis [72]. The proteasome inhibitor bortezomib (Velcade) has demonstrated cytotoxic activity as a single drug or in combination with therapy in clinical studies in cancer cell lines [102]. In addition, the randomized phase II trial of bortezomib alone and bortezomib in combination with docetaxel was valid, and the clear performance of the two treatments was similar to the secondary treatment in NSCLC [103]. More research on cancer is anticipated with bortezomib along with chemotherapy [72].
- 4. Insulin Growth Factor Pathway Inhibition:** The insulin-like growth factor (IGF) pathway plays role in the growth and differentiation of bones and cartilages. It usually has two receptors viz insulin-like growth factor 1 receptor (IGF-1R) and insulin receptor (IR) in addition of having usually three ligands namely IGF-1, IGF-2 and insulin [104]. The insulin-like growth factor 1 receptor is the tyrosine kinase that undergoes homodimerization or heterodimerization with HER2 isoform and insulin receptor. IGF-1R does not appear in a mutated form in cancer as like HER2. Binding of ligand leads to activation of several signaling pathways, including the RAS/RAK/MEK and PI3K/AKT/mTOR pathways. Around up to 70% overexpression of IGF-1R in NSCLC is evidence of dysregulation of IGF signaling in lung cancer [105, 106], where strong signaling leads to drug resistance and ultimately tumor growth [107]. In addition, regulation of IGF-1 is frequently associated with lung cancer risk [108][109]. A phase III study investigating the combination of carboplatin, figitumab, and paclitaxel as first-line therapy in patients with advanced NSCLC was also terminated due to lack of efficacy [110].
- 5. Enhancing apoptosis:** Cancer cells have the ability to escape apoptosis. Bcl-2 is overexpressed in 75%-95% of SCLC and 10%-35% of NSCLC, and shows anti-apoptotic activity[90]. The preclinical data demonstrated that sodium oblimersen is an antisense oligonucleotide that targets Bcl-2 conferring resistance to treatment with radiotherapy, monoclonal antibodies and traditional cytotoxic chemotherapy. A randomized phase II study of oblimersen combined with chemotherapy for the treatment of NSCLC and SCLC is ongoing [111]. Potential small molecule inhibitors of the antiapoptotic proteins Bcl-XL, Bcl-2 and Bcl-w (for example (ABT-737) have been further developed and have shown efficacy as single agents in both SCLC and NSCLC cases [112].
- 6. Heat Shock Protein Inhibition:** Heat shock proteins (HSPs) are the molecular chaperones associated with signal transduction and stability, post-translational folding and activation of several proteins needed for the cell cycle progression. In addition, they are oncogenic chaperones and the inhibition of HSP90 which is the well-known HSP

proteins, causes disruption of oncogenes such as BCR-ABL, HER2 and BRAF, and inhibit many oncogenic signaling pathways [113]. Scientists have identified geldanamycin is an HSP90 inhibitor and have additionally developed several 17-amino acid derivatives, such as 17-AAG, SNX-5422, ganetespib, and retamycin [114].

- 7. Telomerase inhibitor:** Several studies show that during tumorigenesis the telomerase activity gets unregulated and is also prevalent in lung cancer. Telomeres are sequences present at the ends of mammalian chromosomes responsible for the prevention of degradation and loss of many important genes [115]. On each division of the cell, the telomere gradually shortens, thereby limiting the lifespan of the somatic cell. The shortening of telomeres and the ensuing cell death can be overcome by telomerase activity, by stabilizing the telomere length on adding DNA sequences to the telomere end side of chromosomes. The human telomerase has two main components namely the functional telomerase RNA (TERC) and the telomerase reverse transcriptase (hTERT) catalytic subunit. Telomerase activation is considered to play an important role in the immortalization of cells at an early stage of cancer.

Telomerase is ubiquitous in human tumors, whereas its activity is either diminished or absent in normal cells. Although telomerase is silent in normal cells, it is activated in about 80% of NSCLC and about 100% of SCLC cases. Therefore, telomerase represents a promising target in treating lung cancer, and several drug candidates targeting telomerase activity have been developed so far. The drug molecule, GRN163L is a novel antagonist of telomerase that targets RNA region of TERC template. The research has demonstrated that GRN163L can reduce the tumor growth of lung cancer cells *in vivo* significantly [116], and phase I clinical investigation with this molecule is in progress. Recently treatments targeting telomerase are in development, which includes gene therapy, reverse transcriptase inhibitors, and immunotherapy [115].

## V. CANCER STEM CELL-TARGETED STRATEGIES

In addition to important survival and self-renewal mechanisms, recent guidelines on glioblastoma state that people with cancer become resistant to effective radiation therapy. A good way to avoid cancer stem cells versus cytotoxic therapy is to inhibit some kinase checkpoints (e.g. Chk1, Txc2) that can have effect on cell cycle in DNA repairing [117]. Other studies have demonstrated the possibility of using soluble substances such as bone morphogenetic protein as therapeutic targets to induce stem cell differentiation [118]. Approaches to treat specific CSC populations include selection of targets using CSC assays, sensitivity of CSC to different clinical and therapeutic models. Additionally the inhibition of signaling pathways associated with CSC such as Wnt, Hedgehog, and Notch signaling pathways and telomerase inhibition are also the approaches that are useful. Inhibition of the Hedgehog pathway was evaluated with natural product cyclopamine and its significant results lead to the development of a synthetic oral inhibitors with observed activity against basal cell carcinoma, as it was used as lead candidate in drug development [119]. Inhibition in the Notch signaling pathway was potentially demonstrated with  $\gamma$ -secretase inhibitors [59].

## VI. CONCLUSION

Despite the advanced technology, cancer mortality incidence including that of lung cancer has not yet declined. Enormous resources have been employed globally for developing a preventive, diagnostic, and therapeutic approach for lung cancer. Relapse and metastasis in patients are the demerits that occur after traditional cancer therapies, such as surgery, radiation, or chemotherapy. Drug development is the challenging process for scientists as it involves an array of transition from design, screening, animal model and clinical trials to get an effective drug candidate. Natural products and their synthetic derivatives have been well used for many years as a source of promising therapeutic agents in anticancer research. Heterocyclic compounds are the privileged scaffolds that have emerged as a promising agent for designing and developing drugs. They can serve as useful tools to alter the polarity, lipophilicity, and hydrogen-bonding capacity of molecules, resulting in improved pharmacological, physicochemical, pharmacokinetic, and toxicological properties of drug candidates for lung cancer. The synthetic cyclic compounds employed as anticancer drugs imitate natural ligands and substrates to disturb the obscure balance in cells. Molecular hybridization is an innovative and attractive approach that provides a platform for the designing and developing novel drug prototypes with improved pharmacokinetics and pharmacodynamics activity. Currently used anticancer drugs targeting DNA or RNA activity mostly rely on their inhibition against synthesis, transcription factors, and enzymes. The majority of these anticancer drugs display a lack of selectivity and participate in drug resistance, limiting the efficacy of anticancer drugs. However, novel therapeutic strategies are being developed to overcome these complications, which may discover novel anticancer drugs with low toxicity and resistance.

## REFERENCES

- [1] Siegel RL, Miller KD, Wagle NS, Jemal A (2023) Cancer statistics, 2023. *CA Cancer J Clin* 73:17–48. <https://doi.org/10.3322/caac.21763>
- [2] McWilliams A, Mayo J, MacDonald S, et al (2003) Lung Cancer Screening. *Am J Respir Crit Care Med* 168:1167–1173. <https://doi.org/10.1164/rccm.200301-144OC>
- [3] Ahmedin Jemal, DVM, PhD; Taylor Murray; Elizabeth Ward, PhD; Alicia Samuels M, Ram C. Tiwari, PhD; Asma Ghafoor, MPH; Eric J. Feuer, PhD; Michael J. Thun, MD M (2005) Cancer statistics. *A Cancer J Clin* 55:10–30. <https://doi.org/10.1136/bmj.1.6004.280-c>
- [4] Ghosal R, Kloer P, Lewis KE (2009) A review of novel biological tools used in screening for the early detection of lung cancer. *Postgrad Med J* 85:358–363. <https://doi.org/10.1136/pgmj.2008.076307>
- [5] Xiang D, Zhang B, Doll D, et al (2013) Lung cancer screening: From imaging to biomarker. *Biomark Res* 1:4. <https://doi.org/10.1186/2050-7771-1-4>
- [6] Travis WD, Brambilla E, Nicholson AG, et al (2015) The 2015 World Health Organization Classification of Lung Tumors. *J Thorac Oncol* 10:1243–1260. <https://doi.org/10.1097/JTO.0000000000000630>
- [7] Denisenko T V., Budkevich IN, Zhivotovsky B (2018) Cell death-based treatment of lung adenocarcinoma. *Cell Death Dis* 9:117. <https://doi.org/10.1038/s41419-017-0063-y>
- [8] Fontana RS, Sanderson DR, Taylor WF, et al (1984) Early lung cancer detection: Results of the initial (prevalence) radiologic and cytologic screening in the Mayo Clinic Study. *Am Rev Respir Dis* 130:561–565. <https://doi.org/10.1164/arrd.1984.130.4.549>
- [9] Marcus PM, Bergstralh EJ, Fagerstrom RM, et al (2000) Lung Cancer Mortality in the Mayo Lung Project: Impact of Extended Follow-up. *JNCI J Natl Cancer Inst* 92:1308–1316. <https://doi.org/10.1093/jnci/92.16.1308>
- [10] Fontana RS, Sanderson DR, Woolner LB, et al (1986) Lung cancer screening: The mayo program. *J Occup Med* 28:746–750. <https://doi.org/10.1097/00043764-198608000-00038>
- [11] Fontana RS, Sanderson DR, Woolner LB, et al (1975) The Mayo Lung Project for Early Detection and Localization of Bronchogenic Carcinoma: A Status Report. *Chest* 67:511–522.

- <https://doi.org/10.1378/chest.67.5.511>
- [12] Muhm JR, Miller WE, Fontana RS, et al (1983) Lung cancer detected during a screening program using four-month chest radiographs. *Radiology* 148:609–615. <https://doi.org/10.1148/radiology.148.3.6308709>
- [13] Flehinger BJ, Melamed MR, Zaman MB, Heelan RT, Perchick WB MN (1984) Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Memorial Sloan-Kettering study. *Am Rev Respir Dis* 130:555–560
- [14] Melamed MR, Flehinger BJ, Zaman MB, et al (1984) Screening for Early Lung Cancer. *Chest* 86:44–53. <https://doi.org/10.1378/chest.86.1.44>
- [15] Doria-Rose VP, Marcus PM, Szabo E, et al (2009) Randomized controlled trials of the efficacy of lung cancer screening by sputum cytology revisited. *Cancer* 115:5007–5017. <https://doi.org/10.1002/cncr.24545>
- [16] Doria-Rose VP, Marcus PM (2009) Death certificates provide an adequate source of cause of death information when evaluating lung cancer mortality: An example from the Mayo Lung Project. *Lung Cancer* 63:295–300. <https://doi.org/10.1016/j.lungcan.2008.05.019>
- [17] Frost JK, Ball WC, Levin ML, et al (1984) Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study. *Am Rev Respir Dis* 130:549–54. <https://doi.org/10.1164/arrd.1984.130.4.549>
- [18] Killian G (1898) Über direkte Bronchoskopie. *Munch Med Wochenschr* 27:27:844–847
- [19] Navani N, Spiro SG, Janes SM (2009) Mediastinal staging of NSCLC with endoscopic and endobronchial ultrasound. *Nat Rev Clin Oncol* 6:278–286. <https://doi.org/10.1038/nrclinonc.2009.39>
- [20] Navani N, Nankivell M, Lawrence DR, et al (2015) Lung cancer diagnosis and staging with endobronchial ultrasound-guided transbronchial needle aspiration compared with conventional approaches: an open-label, pragmatic, randomised controlled trial. *Lancet Respir Med* 3:282–289. [https://doi.org/10.1016/S2213-2600\(15\)00029-6](https://doi.org/10.1016/S2213-2600(15)00029-6)
- [21] Herth FJF, Eberhardt R, Ernst A (2006) The Future of Bronchoscopy in Diagnosing, Staging and Treatment of Lung Cancer. *Respiration* 73:399–409. <https://doi.org/10.1159/000093369>
- [22] Laurent F, Montaudon M, Corneloup O (2006) CT and MRI of Lung Cancer. *Respiration* 73:133–142. <https://doi.org/10.1159/000091528>
- [23] Qu J, MacAulay C, Lam S, Palcic B (1994) Optical properties of normal and carcinomatous bronchial tissue. *Appl Opt* 33:7397. <https://doi.org/10.1364/AO.33.007397>
- [24] Moghissi K, Dixon K, Stringer MR (2008) Current indications and future perspective of fluorescence bronchoscopy: A review study. *Photodiagnosis Photodyn Ther* 5:238–246. <https://doi.org/10.1016/j.pdpdt.2009.01.008>
- [25] Blandin Knight S, Crosbie PA, Balata H, et al (2017) Progress and prospects of early detection in lung cancer. *Open Biol* 7:170070. <https://doi.org/10.1098/rsob.170070>
- [26] Hayes J, Peruzzi PP, Lawler S (2014) MicroRNAs in cancer: biomarkers, functions and therapy. *Trends Mol Med* 20:460–469. <https://doi.org/10.1016/j.molmed.2014.06.005>
- [27] Takamizawa J, Konishi H, Yanagisawa K, et al (2004) Reduced Expression of the let-7 MicroRNAs in Human Lung Cancers in Association with Shortened Postoperative Survival. *Cancer Res* 64:3753–3756. <https://doi.org/10.1158/0008-5472.CAN-04-0637>
- [28] Skog J, Würdinger T, van Rijn S, et al (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 10:1470–1476. <https://doi.org/10.1038/ncb1800>
- [29] Boeri M, Verri C, Conte D, et al (2011) MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer. *Proc Natl Acad Sci* 108:3713–3718. <https://doi.org/10.1073/pnas.1100048108>
- [30] Bach PB (2007) Computed Tomography Screening and Lung Cancer Outcomes. *JAMA* 297:953. <https://doi.org/10.1001/jama.297.9.953>
- [31] Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T (2014) Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. *Nat Rev Cancer* 14:135–146. <https://doi.org/10.1038/nrc3670>
- [32] Matsushita H, Vesely MD, Koboldt DC, et al (2012) Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature* 482:400–404. <https://doi.org/10.1038/nature10755>
- [33] Medler TR, Cotechini T, Coussens LM (2015) Immune Response to Cancer Therapy: Mounting an Effective Antitumor Response and Mechanisms of Resistance. *Trends in Cancer* 1:66–75. <https://doi.org/10.1016/j.trecan.2015.07.008>
- [34] Zaenker P, Gray ES, Ziman MR (2016) Autoantibody Production in Cancer—The Humoral Immune

- Response toward Autologous Antigens in Cancer Patients. *Autoimmun Rev* 15:477–483. <https://doi.org/10.1016/j.autrev.2016.01.017>
- [35] Mack U, Ukena D, Montemar M, Sybrecht GW (2000) Serum anti-p53 antibodies in patients with lung cancer. *Oncol Rep*. <https://doi.org/10.3892/or.7.3.669>
- [36] Heitzer E, Ulz P, Geigl JB (2015) Circulating Tumor DNA as a Liquid Biopsy for Cancer. *Clin Chem* 61:112–123. <https://doi.org/10.1373/clinchem.2014.222679>
- [37] Newman AM, Bratman S V, To J, et al (2014) An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med* 20:548–554. <https://doi.org/10.1038/nm.3519>
- [38] Sozzi G, Conte D, Leon M, et al (2003) Quantification of Free Circulating DNA As a Diagnostic Marker in Lung Cancer. *J Clin Oncol* 21:3902–3908. <https://doi.org/10.1200/JCO.2003.02.006>
- [39] Abbosh C, Birkbak NJ, Wilson GA, et al (2017) Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature* 545:446–451. <https://doi.org/10.1038/nature22364>
- [40] Hagiwara N, Mechanic LE, Trivers GE, et al (2006) Quantitative Detection of p53 Mutations in Plasma DNA from Tobacco Smokers. *Cancer Res* 66:8309–8317. <https://doi.org/10.1158/0008-5472.CAN-06-0991>
- [41] Yadav VK, DeGregori J, De S (2016) The landscape of somatic mutations in protein coding genes in apparently benign human tissues carries signatures of relaxed purifying selection. *Nucleic Acids Res* 44:2075–2084. <https://doi.org/10.1093/nar/gkw086>
- [42] Kölbl A, Jeschke U, Andergassen U (2016) The Significance of Epithelial-to-Mesenchymal Transition for Circulating Tumor Cells. *Int J Mol Sci* 17:1308. <https://doi.org/10.3390/ijms17081308>
- [43] Williamson SC, Metcalf RL, Trapani F, et al (2016) Vasculogenic mimicry in small cell lung cancer. *Nat Commun* 7:13322. <https://doi.org/10.1038/ncomms13322>
- [44] Labelle M, Hynes RO (2012) The Initial Hours of Metastasis: The Importance of Cooperative Host–Tumor Cell Interactions during Hematogenous Dissemination. *Cancer Discov* 2:1091–1099. <https://doi.org/10.1158/2159-8290.CD-12-0329>
- [45] Yang M-H, Imrali A, Heeschen C (2015) Circulating cancer stem cells: the importance to select. *Chin J Cancer Res* 27:437–49. <https://doi.org/10.3978/j.issn.1000-9604.2015.04.08>
- [46] Hodgkinson CL, Morrow CJ, Li Y, et al (2014) Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. *Nat Med* 20:897–903. <https://doi.org/10.1038/nm.3600>
- [47] Barriere G, Fici P, Gallerani G, et al (2014) Circulating tumor cells and epithelial, mesenchymal and stemness markers: characterization of cell subpopulations. *Ann Transl Med* 2:109. <https://doi.org/10.3978/j.issn.2305-5839.2014.10.04>
- [48] Hofman V, Ilie MI, Long E, et al (2011) Detection of circulating tumor cells as a prognostic factor in patients undergoing radical surgery for non-small-cell lung carcinoma: comparison of the efficacy of the CellSearch Assay™ and the isolation by size of epithelial tumor cell method. *Int J Cancer* 129:1651–1660. <https://doi.org/10.1002/ijc.25819>
- [49] Lou J, Ben S, Yang G, et al (2013) Quantification of Rare Circulating Tumor Cells in Non-Small Cell Lung Cancer by Ligand-Targeted PCR. *PLoS One* 8:e80458. <https://doi.org/10.1371/journal.pone.0080458>
- [50] Campton DE, Ramirez AB, Nordberg JJ, et al (2015) High-recovery visual identification and single-cell retrieval of circulating tumor cells for genomic analysis using a dual-technology platform integrated with automated immunofluorescence staining. *BMC Cancer* 15:360. <https://doi.org/10.1186/s12885-015-1383-x>
- [51] Spiro SG, Hackshaw A (2016) Research in progress—LungSEARCH: a randomised controlled trial of surveillance for the early detection of lung cancer in a high-risk group. *Thorax* 71:91–93. <https://doi.org/10.1136/thoraxjnl-2015-207433>
- [52] Hubers AJ, Prinsen CFM, Sozzi G, et al (2013) Molecular sputum analysis for the diagnosis of lung cancer. *Br J Cancer* 109:530–537. <https://doi.org/10.1038/bjc.2013.393>
- [53] Somers VA, Pietersen AM, Theunissen PH, Thunnissen FB (1998) Detection of K-ras point mutations in sputum from patients with adenocarcinoma of the lung by point-EXACCT. *J Clin Oncol* 16:3061–3068. <https://doi.org/10.1200/JCO.1998.16.9.3061>
- [54] Harnan SE, Tappenden P, Essat M, et al (2015) Measurement of exhaled nitric oxide concentration in asthma: a systematic review and economic evaluation of NIOX MINO, NIOX VERO and NObreath. *Health Technol Assess* 19:1–330. <https://doi.org/10.3310/hta19820>
- [55] McCulloch M, Jezierski T, Broffman M, et al (2006) Diagnostic Accuracy of Canine Scent Detection in Early- and Late-Stage Lung and Breast Cancers. *Integr Cancer Ther* 5:30–39. <https://doi.org/10.1177/1534735405285096>

- [56] Ehmann R, Boedeker E, Friedrich U, et al (2012) Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. *Eur Respir J* 39:669–676. <https://doi.org/10.1183/09031936.00051711>
- [57] Machado RF, Laskowski D, Deffenderfer O, et al (2005) Detection of Lung Cancer by Sensor Array Analyses of Exhaled Breath. *Am J Respir Crit Care Med* 171:1286–1291. <https://doi.org/10.1164/rccm.200409-1184OC>
- [58] Weinstein IB, Joe AK (2006) Mechanisms of Disease: oncogene addiction—a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol* 3:448–457. <https://doi.org/10.1038/nponc0558>
- [59] Larsen JE, Cascone T, Gerber DE, et al (2011) Targeted therapies for lung cancer: clinical experience and novel agents. *Cancer J* 17:512–27. <https://doi.org/10.1097/PPO.0b013e31823e701a>
- [60] Takano T, Fukui T, Ohe Y, et al (2008) EGFR Mutations Predict Survival Benefit From Gefitinib in Patients With Advanced Lung Adenocarcinoma: A Historical Comparison of Patients Treated Before and After Gefitinib Approval in Japan. *J Clin Oncol* 26:5589–5595. <https://doi.org/10.1200/JCO.2008.16.7254>
- [61] Kris MG, Johnson BE, Berry LD, et al (2014) Using Multiplexed Assays of Oncogenic Drivers in Lung Cancers to Select Targeted Drugs. *JAMA* 311:1998. <https://doi.org/10.1001/jama.2014.3741>
- [62] Barlesi F, Mazieres J, Merlio J-P, et al (2016) Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). *Lancet* 387:1415–1426. [https://doi.org/10.1016/S0140-6736\(16\)00004-0](https://doi.org/10.1016/S0140-6736(16)00004-0)
- [63] Shigematsu H, Lin L, Takahashi T, et al (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 97:339–346. <https://doi.org/10.1093/jnci/dji055>
- [64] Pao W, Miller VA, Politi KA, et al (2005) Acquired Resistance of Lung Adenocarcinomas to Gefitinib or Erlotinib Is Associated with a Second Mutation in the EGFR Kinase Domain. *PLoS Med* 2:e73. <https://doi.org/10.1371/journal.pmed.0020073>
- [65] Kobayashi S, Boggon TJ, Dayaram T, et al (2005) EGFR Mutation and Resistance of Non–Small-Cell Lung Cancer to Gefitinib. *N Engl J Med* 352:786–792. <https://doi.org/10.1056/NEJMoa044238>
- [66] Cappuzzo F, Hirsch FR, Rossi E, et al (2005) Epidermal Growth Factor Receptor Gene and Protein and Gefitinib Sensitivity in Non–Small-Cell Lung Cancer. *JNCI J Natl Cancer Inst* 97:643–655. <https://doi.org/10.1093/jnci/dji112>
- [67] Cappuzzo F, Magrini E, Ceresoli GL, et al (2004) Akt Phosphorylation and Gefitinib Efficacy in Patients With Advanced Non-Small-Cell Lung Cancer. *JNCI J Natl Cancer Inst* 96:1133–1141. <https://doi.org/10.1093/jnci/djh217>
- [68] Herbst RS, Prager D, Hermann R, et al (2005) TRIBUTE: A Phase III Trial of Erlotinib Hydrochloride (OSI-774) Combined With Carboplatin and Paclitaxel Chemotherapy in Advanced Non–Small-Cell Lung Cancer. *J Clin Oncol* 23:5892–5899. <https://doi.org/10.1200/JCO.2005.02.840>
- [69] Herbst RS, Giaccone G, Schiller JH, et al (2004) Gefitinib in Combination With Paclitaxel and Carboplatin in Advanced Non–Small-Cell Lung Cancer: A Phase III Trial—INTACT 2. *J Clin Oncol* 22:785–794. <https://doi.org/10.1200/JCO.2004.07.215>
- [70] Werner-Wasik M, Swann S, Curran W, et al (2005) A phase II study of cetuximab (C225) in combination with chemoradiation (CRT) in patients (PTS) with stage IIIA/B non-small cell lung cancer (NSCLC): An interim overall toxicity report of the RTOG 0324 Trial. *J Clin Oncol* 23:7135–7135. [https://doi.org/10.1200/jco.2005.23.16\\_suppl.7135](https://doi.org/10.1200/jco.2005.23.16_suppl.7135)
- [71] Coate LE, Gately K, Barr MP, et al (2006) Phase II pilot study of neoadjuvant cetuximab in combination with cisplatin and gemcitabine in patients with resectable IB-IIIa non small cell lung cancer. *J Clin Oncol* 24:17107–17107. [https://doi.org/10.1200/jco.2006.24.18\\_suppl.17107](https://doi.org/10.1200/jco.2006.24.18_suppl.17107)
- [72] Sun S, Schiller JH, Spinola M, Minna JD (2007) New molecularly targeted therapies for lung cancer. *J Clin Invest* 117:2740–2750. <https://doi.org/10.1172/JCI31809>
- [73] Cardones A, Banez L (2006) VEGF Inhibitors in Cancer Therapy. *Curr Pharm Des* 12:387–394. <https://doi.org/10.2174/138161206775201910>
- [74] Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 82:4–7. <https://doi.org/10.1093/jnci/82.1.4>
- [75] Ferrara N (1999) Molecular and biological properties of vascular endothelial growth factor. *J Mol Med* 77:527–543. <https://doi.org/10.1007/s001099900019>
- [76] Stefanou D, Batistatou A, Arkoumani E, et al (2004) Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in small-cell and non-small-cell lung carcinomas. *Histol Histopathol* 19:
- [77] Jantus-Lewintre E, Sanmartín E, Sirera R, et al (2011) Combined VEGF-A and VEGFR-2 concentrations

- in plasma: Diagnostic and prognostic implications in patients with advanced NSCLC. *Lung Cancer* 74:326–331. <https://doi.org/10.1016/j.lungcan.2011.02.016>
- [78] Dudek AZ, Mahaseth H (2005) Circulating Angiogenic Cytokines in Patients with Advanced Non-Small Cell Lung Cancer: Correlation with Treatment Response and Survival. *Cancer Invest* 23:193–200. <https://doi.org/10.1081/CNV-200055949>
- [79] Kaya A, Çiledag A, Gulbay BE, et al (2004) The prognostic significance of vascular endothelial growth factor levels in sera of non-small cell lung cancer patients. *Respir Med* 98:632–636. <https://doi.org/10.1016/j.rmed.2003.12.017>
- [80] Reck M, von Pawel J, Zatloukal P, et al (2009) Phase III Trial of Cisplatin Plus Gemcitabine With Either Placebo or Bevacizumab As First-Line Therapy for Nonsquamous Non-Small-Cell Lung Cancer: AVAiL. *J Clin Oncol* 27:1227–1234. <https://doi.org/10.1200/JCO.2007.14.5466>
- [81] Sandler A, Gray R, Perry MC, et al (2006) Paclitaxel–Carboplatin Alone or with Bevacizumab for Non-Small-Cell Lung Cancer. *N Engl J Med* 355:2542–2550. <https://doi.org/10.1056/NEJMoa061884>
- [82] Heymach J V., Paz-Ares L, De Braud F, et al (2008) Randomized Phase II Study of Vandetanib Alone or With Paclitaxel and Carboplatin as First-Line Treatment for Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 26:5407–5415. <https://doi.org/10.1200/JCO.2008.17.3138>
- [83] Heymach J V., Johnson BE, Prager D, et al (2006) A phase II trial of ZD6474 plus docetaxel in patients with previously treated NSCLC: Follow-up results. *J Clin Oncol* 24:7016–7016. [https://doi.org/10.1200/jco.2006.24.18\\_suppl.7016](https://doi.org/10.1200/jco.2006.24.18_suppl.7016)
- [84] Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-kinase-AKT pathway in human cancer. *Nat Rev Cancer* 2:489–501. <https://doi.org/10.1038/nrc839>
- [85] Brognard J, Clark AS, Ni Y, Dennis PA (2001) Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. *Cancer Res* 61:3986–3997
- [86] Kawano O, Sasaki H, Endo K, et al (2006) PIK3CA mutation status in Japanese lung cancer patients. *Lung Cancer* 54:209–215. <https://doi.org/10.1016/j.lungcan.2006.07.006>
- [87] Samuels Y, Wang Z, Bardelli A, et al (2004) High Frequency of Mutations of the PIK3CA Gene in Human Cancers. *Science* (80- ) 304:554–554. <https://doi.org/10.1126/science.1096502>
- [88] Soria JC, Shepherd FA, Douillard JY, et al (2009) Efficacy of everolimus (RAD001) in patients with advanced NSCLC previously treated with chemotherapy alone or with chemotherapy and EGFR inhibitors. *Ann Oncol* 20:1674–1681. <https://doi.org/10.1093/annonc/mdp060>
- [89] Downward J (2003) Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 3:11–22. <https://doi.org/10.1038/nrc969>
- [90] Sekido Y, Fong KM, Minna JD (2003) Molecular Genetics of Lung Cancer. *Annu Rev Med* 54:73–87. <https://doi.org/10.1146/annurev.med.54.101601.152202>
- [91] Mascaux C, Iannino N, Martin B, et al (2005) The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer* 92:131–139. <https://doi.org/10.1038/sj.bjc.6602258>
- [92] Shigematsu H, Gazdar AF (2006) Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int J Cancer* 118:257–262. <https://doi.org/10.1002/ijc.21496>
- [93] Massarelli E, Varella-Garcia M, Tang X, et al (2007) KRAS Mutation Is an Important Predictor of Resistance to Therapy with Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non-Small-Cell Lung Cancer. *Clin Cancer Res* 13:2890–2896. <https://doi.org/10.1158/1078-0432.CCR-06-3043>
- [94] Isobe T, Herbst RS, Onn A (2005) Current Management of Advanced Non-Small Cell Lung Cancer: Targeted Therapy. *Semin Oncol* 32:315–328. <https://doi.org/10.1053/j.seminoncol.2005.02.016>
- [95] Takahashi T, Nau MM, Chiba I, et al (1989) p53: A Frequent Target for Genetic Abnormalities in Lung Cancer. *Science* (80- ) 246:491–494. <https://doi.org/10.1126/science.2554494>
- [96] Hainaut P (1998) IARC Database of p53 gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. *Nucleic Acids Res* 26:205–213. <https://doi.org/10.1093/nar/26.1.205>
- [97] Takahashi T, Carbone D, Takahashi T, et al (1992) Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. *Cancer Res* 52:2340–3
- [98] Roth JA, Nguyen D, Lawrence DD, et al (1996) Retrovirus-mediated wild-type P53 gene transfer to tumors of patients with lung cancer. *Nat Med* 2:985–991. <https://doi.org/10.1038/nm0996-985>
- [99] Kondo M, Ji L, Kamibayashi C, et al (2001) Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells. *Oncogene* 20:6258–6262. <https://doi.org/10.1038/sj.onc.1204832>

- [100] Ji L, Nishizaki M, Gao B, et al (2002) Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities in vitro and in vivo. *Cancer Res* 62:2715–2720
- [101] Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 3:415–428. <https://doi.org/10.1038/nrg816>
- [102] Adams J, Palombella VJ, Sausville EA, et al (1999) Proteasome inhibitors: A novel class of potent and effective antitumor agents. *Cancer Res* 59:2615–2622
- [103] Fanucchi MP, Fossella F V., Belt R, et al (2006) Randomized Phase II Study of Bortezomib Alone and Bortezomib in Combination With Docetaxel in Previously Treated Advanced Non–Small-Cell Lung Cancer. *J Clin Oncol* 24:5025–5033. <https://doi.org/10.1200/JCO.2006.06.1853>
- [104] Maki RG (2010) Small Is Beautiful: Insulin-Like Growth Factors and Their Role in Growth, Development, and Cancer. *J Clin Oncol* 28:4985–4995. <https://doi.org/10.1200/JCO.2009.27.5040>
- [105] Nahta R, Yu D, Hung M-C, et al (2006) Mechanisms of Disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nat Clin Pract Oncol* 3:269–280. <https://doi.org/10.1038/ncponc0509>
- [106] Gong Y, Yao E, Shen R, et al (2009) High Expression Levels of Total IGF-1R and Sensitivity of NSCLC Cells In Vitro to an Anti-IGF-1R Antibody (R1507). *PLoS One* 4:e7273. <https://doi.org/10.1371/journal.pone.0007273>
- [107] Morgillo F, Kim W-Y, Kim ES, et al (2007) Implication of the Insulin-like Growth Factor-IR Pathway in the Resistance of Non–small Cell Lung Cancer Cells to Treatment with Gefitinib. *Clin Cancer Res* 13:2795–2803. <https://doi.org/10.1158/1078-0432.CCR-06-2077>
- [108] Han J-Y, Choi BG, Choi JY, et al (2006) The prognostic significance of pretreatment plasma levels of insulin-like growth factor (IGF)-1, IGF-2, and IGF binding protein-3 in patients with advanced non-small cell lung cancer. *Lung Cancer* 54:227–234. <https://doi.org/10.1016/j.lungcan.2006.07.014>
- [109] Yu H, Spitz MR, Mistry J, et al (1999) Plasma Levels of Insulin-Like Growth Factor-I and Lung Cancer Risk: a Case-Control Analysis. *JNCI J Natl Cancer Inst* 91:151–156. <https://doi.org/10.1093/jnci/91.2.151>
- [110] Jassem J, Langer CJ, Karp DD, et al (2010) Randomized, open label, phase III trial of figitumumab in combination with paclitaxel and carboplatin versus paclitaxel and carboplatin in patients with non-small cell lung cancer (NSCLC). *J Clin Oncol* 28:7500–7500. [https://doi.org/10.1200/jco.2010.28.15\\_suppl.7500](https://doi.org/10.1200/jco.2010.28.15_suppl.7500)
- [111] Herbst RS, Frankel SR (2004) Oblimersen Sodium (Genasense bcl-2 Antisense Oligonucleotide). *Clin Cancer Res* 10:4245s–4248s. <https://doi.org/10.1158/1078-0432.CCR-040018>
- [112] Oltersdorf T, Elmore SW, Shoemaker AR, et al (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 435:677–681. <https://doi.org/10.1038/nature03579>
- [113] Chirieac LR, Dacic S (2010) Targeted Therapies in Lung Cancer. *Surg Pathol Clin* 3:71–82. <https://doi.org/10.1016/j.path.2010.04.001>
- [114] Uehara Y (2003) Natural Product Origins of Hsp90 Inhibitors. *Curr Cancer Drug Targets* 3:325–330. <https://doi.org/10.2174/1568009033481796>
- [115] Shay JW, Wright WE (2006) Telomerase therapeutics for cancer: challenges and new directions. *Nat Rev Drug Discov* 5:577–584. <https://doi.org/10.1038/nrd2081>
- [116] Dikmen ZG, Gellert GC, Jackson S, et al (2005) In vivo Inhibition of Lung Cancer by GRN163L: A Novel Human Telomerase Inhibitor. *Cancer Res* 65:7866–7873. <https://doi.org/10.1158/0008-5472.CAN-05-1215>
- [117] Bao S, Wu Q, McLendon RE, et al (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444:756–760. <https://doi.org/10.1038/nature05236>
- [118] Piccirillo SGM, Reynolds BA, Zanetti N, et al (2006) Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature* 444:761–765. <https://doi.org/10.1038/nature05349>
- [119] Von Hoff DD, LoRusso PM, Rudin CM, et al (2009) Inhibition of the Hedgehog Pathway in Advanced Basal-Cell Carcinoma. *N Engl J Med* 361:1164–1172. <https://doi.org/10.1056/NEJMoa0905360>



