IN SILICO ANALYSIS OF EGFR ANTAGONIST IN CANCER TREATMENT

A Project Report Submitted

In Partial Fulfillment of the Requirements

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BACHELOR OF PHARMACY

by

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List of abbreviations

EGFR: Epidermal Growth Factor Receptor

RKT: Tyrosine Kinase Receptor

TGF: Transforming Growth Factor

EGF: Epidermal Growth Factor

GLU: Glutamic Acid

MAPK: Mitogen Activated Protein Kinase

MAbs: Monoclonal Antibody

NSCLC: Non-Small Cell Lung Cancer

SCLC: Small Cell Lung Cancer

ECM: Extra Cellular Matrix

CNS: Central Nervous System

GALGOTIAS UNIVERSITY

CERTIFICATE

This is to certify that project work entitled **"In Silico Analysis Of EGFR Antagonist In Cancer Treatmen**t"done by Ms Sanober Amir submitted to Department of Pharmacy, is a bonafide research work done by Ms Sanober Amir under the supervision and guidance of Ms. Awaneet Kaur**,** Assistant Professor, School of Medical and Allied Sciences, Greater Noida. The work is completed and ready for evaluation in partial fulfillment for the award of Bachelor of Pharmacy during the academic year 2021-2022.The project report has not formed the basis for the award of any Degree/Diploma/Fellowship or other similar title to any candidate of any University.

Date:

Prof. Pramod Kumar Sharma
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BONAFIDECERTIFICATE

This to certify that the project work entitled **"In Silico Analysis Of EGFR Antagonist In Cancer Treatment"**is the bonafide research work done by Ms. Sanober Amir, who carried out the research work under my supervision and guidance for the award of Bachelor of Pharmacy under Galgotias University, Greater Noida during the academic year 2021-2022. To the best of my knowledge the work reported herein is not submitted for award of any other degree or diploma of any other Institute or University.

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DECLARATION

I hereby declare that the work embodied in this project report entitled **"In Silico Analysis Of EGFR Antagonist In Cancer Treatment"** in Partial fulfillment of the requirementsfor the award of Bachelor of Pharmacy, is a record of original and independent research work done by me during the academic year 2021-22 under the supervision and guidance of Ms. Awaneet Kaur**,** Assistant Professor, School of Medical and Allied Sciences, Galgotias University, Greater Noida.I have not submitted this project for award of any other degree or diploma of any other Institute or University.

Date: (Ms. Sanober Amir)

Place: Greater Noida(U.P) **Name and Signature of candidate**

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Ms. Sanober Amir

Abstract

EGFR acts as promising cancer target. Monoclonal antibodies addressing ligand binding extracellular domain, as well as low molecular weight blockers of receptor's tyrosine kinase, used mainly these days in clinical development. Such compounds block ligand induced target cell activation & downstream ixignaling, causing arrest of cell cycle, cell death & angiogenesis blockage. EGFR regulates epithelial tissue growth and homeostasis in a physiological sense. In pathological circumstances, such as lung and breast cancer and glioblastoma, EGFR acts as carcinogenesis driver. The most obvious reason for abnormal EGFR activation for cancer are point mutations and amplification. The first growth factor receptor to be put forward as a cancer therapeutic target was EGFR. Non–small lung cancer cell, neck and head squamous-cell carcinoma, pancreatic cancer & colorectal cancer are among the four metastatic epithelial malignancies for which EGFR antagonists are currently accessible. Treatments ix ignaling EGFR-tyrosine kinase blockers, such as gefitinib & EGFR-neutralizing antibodies, such as bevacizumab and cetuximab, have both been effective in reducing lung cancer. Gefitinib were particularly useful as second- and third-line therapy after chemotherapies. Combining TK inhibitors alongside chemotherapeutics like pemetrexed & docetaxel resulted in notable improvements in overall and progression-free survival in clinical trials of phase 2/3. Combining tyrosine kinase blockers to EGFR-targeted antibodies were also found to be a successful treatment for lung cancer in phase 1 & 2 clinical trials. Docking against EGFR tyrosine kinase with the drug erlotinib shows high binding enery with the catalytic residue of egfr tyrosin kinse which can be a potential drug against lung cancer.

1. INTRODUCTION

1.1. Cancer: Cancer is a disease that develops when abnormal cells divide uncontrollably in one part of the body and invade surrounding tissues. Cancer is caused by a series of gene mutations that disrupt the activities of the cell. The role of chemical compounds in the creation of gene mutations and cancer cells is well established[1].

1.2. ROLE OF EGFR IN CANCER: Epidermal Growth Factor Receptor (EGFR) is a transmembrane target cell belongs to a family of four related proteins [2]. Each receptor can be targeted by ten distinct ligands. When ligands binds on single-chain EGFR, it produces a dimer, which causes receptor autophosphorylation and tyrosine kinase activity inside the cell. Autophosphorylation can cause tumor cell proliferation, death suppression, invasive and metastasis activation, and cancer induced neovascularization stimulation [3,4].

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Uncontrolled production of certain chemicals which enhance cell growth , increased expression of specific proteins on cell membranes (growth factor receptors) to which growth factors selectively bind can give cancer cells the ability to proliferate autonomously and dysregulatedly. Both activities set off a cascade of intracellular signals that eventually lead to cancer cell proliferation, angiogenesis, and metastasis [5]. The majority of human epithelial malignancies are 1ignaling11ed by functional activation of epidermal growth factor receptor (EGFR) family growth factors and receptors. Because of this, EGFR was first to be identify as a cancer therapy. For the treatment of four metastatic epithelial malignancies, four EGFR antagonists are now available. Non–small cell of lung tumor, squamous-cell carcinoma of neck & head, pancreatic

cancer, colorectal cancer & are all cancers that affect the digestive system, after 20 years of pharmacological development. The utility of EGFR antagonists in the therapy of cancer in its early stages is less well understood.EGFR inhibitors' methods of action, clinical proof of their anticancer effectiveness, & current, or contested, clinical problems around their appropriate usage of treatment in cancer patients [2].

EGFR is a receptor tyrosine kinase(RTK) that regulates epithelial cell function. It is related to ErbB family of RTKs [6]. EGFR commonly mutated and/or excessive expression on varities of malignancies, it is used of a variety of tumors medicines now used [7].

EGFR is an important player at epithelial cancer, and its activity promotes tumour growth, invasion, and spread. EGFR is a RKT that transmits a growth-inducing signal to cells triggered by an EGFR ligand (such as TGF and EGF). The supply of these ligands in normal tissues is tightly controlled to ensure that kinetics of cell growth closely match the tissues' homeostasis requirements. In cancer, afterall EGFR is persistently stimulated indefinitely because of extended creation of EGFR ligands into tumour microenvironment or mutation in EGFR which keeps the receptor active. Tumors that overexpress TGF or EGFR have a more aggressive nature, which is often linked to a poor prognosis. EGFR has, predictably, been a primary target for therapeutic intervention. The effects of the EGFR are interpreted within the context of ligand- and kinase-dependent activation, often known as the "canonical" EGFR signalling pathway [8]. However, new functions have lately been discovered, both kinase dependent and independent. They show that the EGFR has unanticipated functions, such as regulating autophagy and metabolism [9]. Cellular and environmental stresses typically induce noncanonical activities. Many of these 'stress pathways' trigger in cancerous cells to give them a advantage in survival & resistance in therapy [9,10]. This has directed to the hypothesis that addressing EGFR and stress pathways at the same time could give a therapy opening for cancer [11].

Oncogenic signalling pathways cause cancer cells to undergo metabolic reprogramming, which promotes tumour growth [12]. EGFR signals have been connected for regulating of various metabolic processes important for cancer cell growth, ranging from fatty acid and pyrimidine synthesis to glucose catabolism [13,14]. Egfr supports the metabolic pathway the two directly and indirectly through phosphorylating rate-limiting enzymes [15,16] & stimulating the MYC transcription factor and the AKT signalling cascade [17,13,14,18,19,20].

The PI3K/AKT-dependent nuclear transfer of sterol regulative protein is increased by EGFRvIII, In glioblastoma multiforme, element binding protein1(SREBP-1) & because of low-frequency lipoprotein receptor(LDLR). Rise in LDLR permits cholesterol to be absorbed without the negative feedback regulation [13]. Because of them cells rely over cholesterol signalling and are very susceptible to blockers of fatty acid and cholesterol production, this constitutes a site of metabolic vulnerability [21].

Moreover, EGFR recently discovered via direct phosphorylate or 3 ignaling stearoylCoA desaturase-1 (SCD1), leading to an increase in monounsaturated fatty acid synthesis [16]. SDC1 phosphorylation is combined with a less outcome in glioblastoma multiforme patients[16]. The Warburg effect, an increase in glycolysis in the presence of oxygen, is another most well-study metabolic shifts at tumor cells. The enthusiastic uptake of glucose by cancer cells is characterised by enhanced membrane expression location about glucose transporter, primarily GLUT1 and GLUT3 [22].Intracellular glucose is converted to pyruvate, which is preferentially transformed to lactate in cancer cells [12].

The EGFR is found that promotes aerobic glycolysis via a variety of methods, both kinase-dependent and kinase-independent.Interaction about EGFR with SGLT1 at cell surface signaling the sodium-glucose cotransporter, enhancing glucose inflow [23]. When cells are grow in the occurance of low glucose concentrations, this kinase-independent activity gives survival benefits, allowing them to avoid autophagic cell death [23]. EGFR regulate manufacturing of Phosphorylation of Pyruvate Kinase M2 (PKM2) , hexokinase (HK1), 2 glycolytic enzymes which catalyse important steps at the system, enhancing aerobic glycolysis on cells of breast cancer in response to EGF stimulation [15]. One important'advese effect' of advanced aerobic glycolysis helps in creating maximum quantities of lactate, which suppresses the cytotoxic activity of T cells in malignant tumours, allowing them to evade the immune system [15]. Deregulated signalling is found towads signalling GLUT1 on cell surface in lung adenocarcinoma cells with oncogenic EGFR mutations by activating the PI3K/AKT/mTOR pathway [18]. Similarly, it is known that activation of AKT in lymphoid cells that responsed to cytokine stimulation prevents GLUT1 endocytosis [24,25]. AKT phosphorylates constrain the thioredoxin-interacting protein (TXNIP), endocytic adaptor involved to GLUT1 CME, according to new research [26,27], suggest that this is the working mechanism on lung cancer

cells with EGFR mutations, suppress of PI3K/AKT/mTOR pathway reduces glycolytic flux, limiting viability [18]. In keeping along these findings, mixed inhibition of EGFR and glycolysis is found directed towards decrease triple-negative breast cancer cell proliferation in a synergistic manner, demonstrating importance about EGFR signalling in cancer cell metabolism [15].

2. DEVELOPMENT OF EGFR ANTAGONIST IN CANCER THERAPY

To summarise our understanding of EGFR signalling, we may break it down into three levels: cell surface, intracellular signalling networks that result in gene transcription and changes in molecular activity, as well as cellular responses [28].

On the cell surface, the earliest ligand-receptor & receptor-receptor communication takes place. ErbB receptors that made up of binding of ligand domains on the outside, transmembrane segments, and intracellular tyrosine kinase domain of protein along carboxyl terminal segment. ErbB receptors can be stimulated in many ways. At physiological position, number of EGFR family ligands stimulate the creation for homo or heterodimeric complexes with four ErbB receptors, allowing signal diversification & amplification [28]. In tumour cells, there are other ways to activate these receptors. To begin with, tumor-induced receptor overexpression could lead directed towards ligand-independent receptor dimerization. In few cancers, like as glioblastoma, mutant forms of the EGFR caused by rearrangements of gene produce ligand-independent constitutive receptor activation and faulty receptor downregulation [29]. G-protein–coupled receptor stimulation activates EGFR by cleaving membrane-bound EGF ligands with metalloproteinases, suggesting which are heterologous ligand-dependent mechanisms that also at work[30].The urokinase plasminogen receptor has been shown to activate EGFR, recently discovered to be ligand-independent [31]. These data show that tumour cells may have other EGFR activation pathways in addition to receptor overexpression, mutations, and autocrine ligand production.

Tyrosine autophosphorylation or stimulation of intrinsic receptor protein tyrosine kinase arise at the signal-processing level. Several intracellular substrates are recruited and phosphorylated as a result of these events, as docking & adaptive fragments attaching at particular phosphotyrosine sites at receptor molecules [32].The Ras-Raf-MAPkinase pathway is a significant downstream

signalling pathway for the ErbB family [33]. Ras activation sets in motion a multistep phosphorylation cascade that includes MAPKs, ERK1, and ERK2 [34]. In laboratory investigations, the transcription of molecules involved in cell proliferation, survival, and transformation is influenced by ERK1 and ERK2 [34]. Phosphatidylinositol 3-kinase (PI3K) or downstream protein-serine or threonine kinase Akt are other significant targets in EGFR signaling[35,36]. Akt sends out signals that set off a chain of events that range from cell survival and motility to cell growth and proliferation [36]. Protein kinase C and Jak/Stat are part of the stress-activated protein kinase cascade, is another avenue for signalling. The activation of these pathways results in diverse transcriptional programmes in the nucleus, which regulate cell differentiation, life (or death), mobility, penetration, adherence, and tissue regeneration are all examples of cellular functions [28]. Nearly 20 years ago, J.M., one of the writers, and his collaborators recommended EGFR as a cancer therapeutic target [37,38]. This hypothesis' logic has been summarized [39]. EGFR is typically overexpressed in human malignancies, as previously stated. Malignancies of the breast, lung, and glioblastoma, as well as cancers of squamous cell, bladder cancer, colorectal cancer, ovarian carcinoma, and prostate cancer, are examples [40]. Overexpression has the potential to increase by a factor of a thousand or more. With the exception of glioblastomas, gene amplification is not a common occurrence in malignancies. Furthermore, as previously mentioned, a mutant form of the EGFR vIII receptor with a loss in the peripheral region causes constitutive activation of its tyrosine kinase in some glioblastomas [29,41,42]. Second, increasing EGFR expression has been linked to a worsening clinical results at variety of cancers, that includes bladder, breast, lung, squamous cell cancers [39,41,43]. Thirdly, increasing receptor contents are frequently linked to boost ligand manufacture by the same tumour cells, being transforming growth factor alpha [40,43,44].This creates a favourable environment for receptor activation via an autocrine stimulatory route.

Early research showed that monoclonal antibodies (MAbs) targeted against EGFR which disrupt binding affinity towards protein suppressed the growth of cancerous cells with elevated protein levels in culture and nude mice xenografts [37,38]. Anti-EGFR MAbs from other companies confirmed these findings.

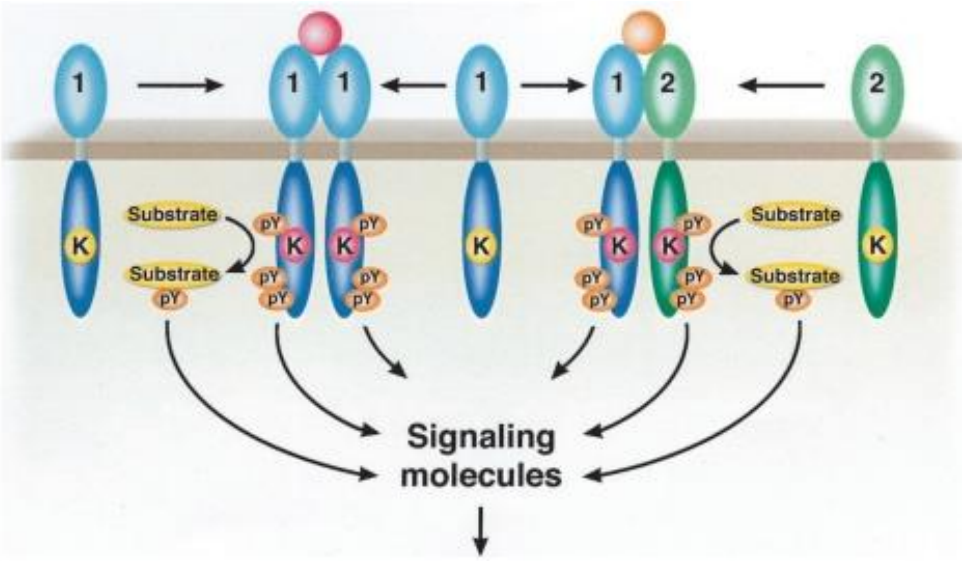

Fig. 1: .Activation mechanisms of receptors. Dimerization activates egfr & members of the target cell family (HER2/3/4).The techniques of complex formation, protein overexpression, and transactivation all promote the formation of receptor base pairs. (heterodimerization). The intrinsic protein tyrosine kinase activity is activated during receptor dimerization, resulting in tyrosine autophosphorylation. Several intracellular substrates are recruited and phosphorylated as a result of these processes, resulting in mitogenic signalling and other cellular functions [28].

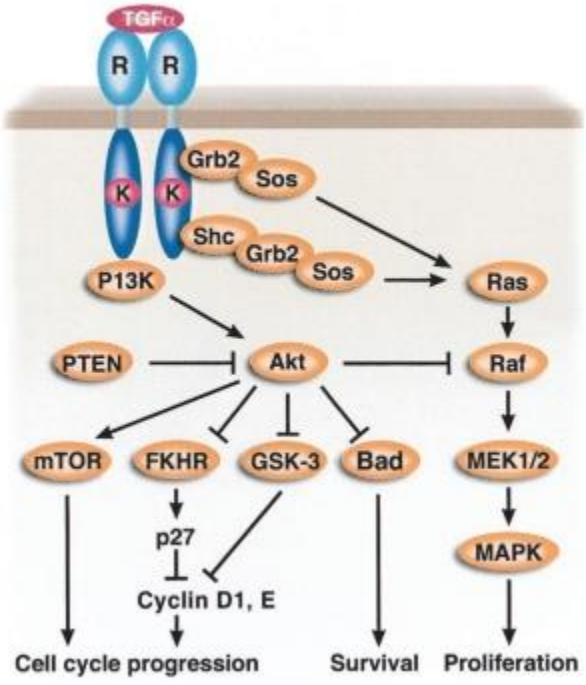

Fig. 2: Signaling by egfr. The stimulation of important intracellular signalling mechanisms that promote cancer by regulating gene transcription, cell growth, and a variety of cellular responses behaviours is the result of interactions between ligands and receptors [39].

Anti-EGFR medicines were originally developed in the 1980s [38]. In phase 3 trials, two kinds of EGFR antagonists were found to be effective and are presently in a therapeutic setting: small-molecule EGFR tyrosine kinase inhibitors and anti-EGFR monoclonal antibodies [45,46,47,48,39].

Anti-EGFR monoclonal antibody like cetuximab interacts with EGFR's dormant extracellular domain and compete with it for receptor binding by blocking the binding of ligand area, and by that prevent EGFR tyrosine kinase stimulation caused by ligand [45,46,49]. Smaller molecule of EGFR tyrosine kinase blockers like erlotinib & gefitinib participate alongside ATP for binding at EGFR tyrosine kinase intracellular catalytic domain, inhibits the EGFR downstream signaling & autophosphorylation. Anti-EGFR monoclonal antibody is utmost selective for EGFR as they are recognised completely. Furthermore, distinct smaller molecules of EGFR tyrosine kinase blockers block tyrosine kinase diverse growth factor receptors, containing EGFR group members or VEGR (Vascular Endothelial Growth Factor) receptor. Several EGFR tyrosine kinase blockes which do not reversible are now into clinical trials [45,46,48]. Anti-EGFR monoclonal antibody & small moleculed EGFR tyrosine kinase blockers contain different modes of action, pharmacologic effects, and range of activity, which may be crucial for clinical activity [50].

Pharmacologic and Functional Inhibitors of EGFR Characteristics [51].		
Features	**Blocking of Monoclonal Antibodies**	**Small Molecules of Tyrosine Kinase blockers**
Administration route	Intravenous (IV) (once in a week or in every two weeks)	Oral (usually regular continuous dosing)
Target selectivity	Exclusively for the EGFR gene	A few EGFR tyrosine kinase blockers also block another growth factor receptors; EGFR tyrosine kinase blockers are relatively selective; they could inhibits one or all EGFR family receptors (for example, dual EGFR & VEGFR inhibitors).
Mechanism of interference with EGFR activation	Bind the extracellular part of the receptor, occluding the ligand area and inhibiting ligand binding and receptor dimerization (cetuximab)	Mostly reversible; EGFR tyrosine kinase blockers which are irreversible and used in clinical development. Binds to intracellular region of receptor into tyrosine kinase domain, often through competing with ATP and preventing receptor autophosphorylation; most of them are reversible.
Cellular effects of egfr inhibition	Actually hinder cancer cell proliferation, angiogenic growth factor (VEGF) synthesis, tumor-induced angiogenesis, and cancer cell invasions (G1 phase arrest); enhance cytotoxic medication and radiotherapy antitumor effect**.**	Adversely impact cancer cell proliferation (G0–G1 phase arrest), VEGF synthesis, tumor-induced angiogenesis, and cancer cell invasions; enhance cytotoxic medication and radiotherapy antitumor activity.
Internalization, down-regulation,	Yes	No (even though EGFR tyrosine

and degradation of EGFR		kinase blockers might result in EGFR degradation & subsequent EGFR downregulation).
Inhibition to egfr-dependent intracellular signaling	Yes	Yes
Activity against proteins of mutant EGFR	Anti-EGFR monoclonal antibody binds with EGFR extracellular domain, therefore mutations in the domain of egfr tyrosin kinase are likely; mutations at the EGFR extracellular domain are unknown.	Yes,because such EGFR mutant protein binds with higher-affinity smaller molecules of EGFR tyrosine kinase blockers like erlotinib or gefitinib, yes as most EGFR tyrosine kinase domain mutations (mutations at codons 746–750 at exon 19 and L858R in exon 21); no for gefitinib- or erlotinib-acquired.
Activation of host immune response	Yes, antibody-dependent cytotoxicity may have a role in few anti EGFR monoclonal antibodies' anticancer activities, likely cetuximab; nevertheless, panitumumab has shown no antibody-dependent cytotoxicity.	No

Table 1: Pharmacologic and functional inhibitors of EGFR characteristics

3. ROLE OF EGFR IN LUNG CANCER

Lung cancer progresses due to uncontrolled cellular proliferation, which causes normal cells to change into cancerous ones [52]. The EGFR family of growth factors has been identified as a crucial player in the development and spread of lung cancer [53]. Secretion of growth factors function through specialised signal transduction mechanism transport biological details from EGFR to lung cells because to their inability to permeate cell membrane [54]. These signalling events are triggered by autocrine, paracrine, or both autocrine and paracrine pathways [54,55].

Finally, signalling cascades enhance lung metastasis in addition to cell proliferation and development [53]. In both NSCLC and SCLC, EGFR- RTK (Receptor Tyrosine Kinase plays crucial function for starting and activating signalling events [56].

EGFR activation is also influenced by genetic variables [57]. In lung tumours, mutations in the EGFR affects autocrine and inducible secretion of growth factor & stimulation [57]. In the lung neoplasms, mutations of EGFR disrupt a variety of growth factor signalling mechanisms, causing severe lung carcinogenesis & cancer growth [58]. Bronchioloalveolar cell carcinoma (BAC), which is caused by an EGFR mutation, has been found to be a prevalent type of NSCLC [59,60]. In a study of 120 adenocarcinoma patients in Japan, almost half of them exhibited non-mucinous BAC [61]. Approximately 80% of BAC patient had substantial EGFR gene mutations [62]. This BAC category, which is caused by an EGFR tyrosine kinase mutation, includes high number of lung cancer patients who likely to be treated with inhibitors of EGFR tyrosine kinase [63]. Papillary solid, acinar, lepidic, papillary mucinous and micropapillary tumours are some of the other characteristics of lung cancer [64-65].

3.1. EGRF: Mechanism of action into Lung Cancer

EGFR related to the member of transmembrane receptor & has three distinct regions [66]. EGF, Heparin-targeting or EGF-like growth factor (HB-EGF), transforming growth factor (TGF), betacellulin, epiregulin, and amphiregulin are all EGFR ligands that the extracellular ligand-binding domain interacts to [3,27]. EGF is the most prominently up-regulated EGFR ligand in lung cancer [58,67]. The ligand-binding domain of EGFR is linked to the intracellular tyrosine kinase signalling domain through the transmembrane domain [66]. Following ligand interaction, EGFR auto-dimers & hetero-dimers along another HER/erbB family tyrosine kinases, including HER1 (EGFR/erbB1), HER2 (neu, erbB2), HER3 (erbB3), and HER4 (erbB4) [68]. Ligand & dimerization binding required for EGFR signalling and targeted activities to occur [53,69]. The dimeric form of EGFR inhibits the intracellular tyrosine kinase domain's auto-inhibitory function, promoting tyrosine phosphorylation and downstream signalling [66,70]. In lung cancer, signalling is initiated by ATP-mediated autophosphorylation of tyrosine, which largely promotes mammalian mechanistic target to rapamycin (mTOR)-serine/threonine protein kinase pathway

[71]. In addition to the EGF ligand, up-regulated amphiregulin are linked to a poor prognosis and lower survival rates in NSCLC [72]. There have been cases of progression and metastasis of severe lung cancer due to elevated EGF and amphiregulin ligands, which result in bronchial lesions and subsequent malignant growths [73,74].

The Ras/Mitogen activated Protein Kinase (MAPK) & phosphatidylinositol-3 kinase/Akt (PI3K/AKT) mechanism are activated through EGFR in the lung tumor growth [56]. In SCLC and NSCLC, PI3K/AKT,proliferative signalling pathway it encourage cellular multiplication, later inhibits cell death[56]. Lung cancer cells that exit the tumors development site enter the lymphatic circulation during the metastatic process [75]. The malignant cells spread to distant places via systemic blood flow, where they multiply and expand into metastatic colonies [76]. Angiogenesis are shown to play a critical role to release and translocation in malignant cell from tumours [77]. Activated-EGFR causes the extracellular matrix (ECM) of lung tissues to break down, resulting in increased blood flow to tumour blood vessels via angiogenesis [76,77]. Increased expression of factors that promote angiogenesis, includes VEGF, basic- Interleukin-8, fibroblast growth factor, and platelet-derived endothelial cell growth factor, is also triggered by activated-EGFR [78].

3.2. Targeting EGFR in lung cancer

EGFR proved that it is a unique target in lung cancer therapy through extensive study [58]. Two basic strategies for restricting EGFR function have been proposed: inactivating intracellular TK signalling and using neutralising antibodies against EGFR and its ligands [79,80]. Erlotinib & gefitinib these two for lung cancer, the most well-studied EGFR-TK inactivators [81,82]. Monoclonal antibodies that disrupt EGFR function are cetuximab and bevacizumab [56,83]. These EGFR inhibitors are shown to reduce malignance of lung cells, increase cell death, and decrease lung cancer metastasis [56,84]. In preclinical investigations, these EGFR inhibitors were particularly effective at slowing the progression of lung cancer [85,86]. Cetuximab, in conjunction with radiation and chemotherapy, caused a synergistic or additive effect increase at the death in the cells of lung cancer, in vitro and in vivo animal models of lung cancer [87]. Several Phase I and II clinical studies found that cetuximab, either alone or in combination with chemotherapy, provided some alleviation to lung cancer patients [88]. Cetuximab had a therapeutic effect comparable to chemotherapy in pre-treated recurrent lung cancer patients [87].

Cetuximab, when combined with chemotherapeutic drugs, had a stronger effect [88,89]. In 86 lung cancer patients, treatment with standard chemotherapeutic medicines, cisplatin and vinorelbine, combined with cetuximab demonstrated significant improvements and increased odds of survival compared to treatment with the medications alone [90]. When compared to cetuximab, the EGFR blockers erlotinib and gefitinib, considerd more passable inside cancerous cells [81]. Skin itches, stomach disturbances, hand-foot syndrome, tiredness, coagulation problems, and hemoptysis were the most common side effects of erlotinib and gefitinib when given orally [91,92]. In NSCLC patients, clinical trials by the use of EGFR blockes, particularly gefitinib, showed a 20% success rate & 40% symptomatic improvement [82,93]. However, in Phase-III clinical trials, gefitinib monotherapy failed to improve survival rates [94]. A combination of gefitinib and other chemotherapies failed to provide any benefit in this experiment [94]. Erlotinib was more effective in this condition, as it demonstrated prospective benefits in combination with other chemotherapies, also in Phase III double-blind clinical studies of NSCLC [95].

Erlotinib and gefitinib these are considered to be efficacious to patients with EGFR mutations in clinical trials [63,96]. Erlotinib and gefitinib does not address threonine to methionine mutations in the EGFR gene at codon 790 of exon 20 [97,98]. The medicines, on the other hand, might be designed to specifically point on mis-sense and in-frame mutations at exons 18-21 of the EGFR-TK domain, which have been linked on lung cancer progression and metastasis [63,86,96]. These EGFR-TK medicines improved lung cancer survival and longevity in patients with the aforementioned EGFR mutations [85,99]. Despite this, erlotnib and gefinib resistance has been identified as a key issue in individuals receiving longer treatment after reappearance [97,98]. This EGFR-TK inhibitor resistance was typically caused by a mutation in codon 790 at the exon 20 regions of EGFR, which prevented these medicines from binding [97,98]. Therapeutics that inhibited EGFR with interacting sites other than specific codon sites appeared to be critical for these patients [97,98].

4. TARGETING OF SPECIFIC EGFR INHIBITORS

A. ERLOTINIB - Erlotinib is widely-known treatment for metastatic lung cancer that reduces tyrosine phosphorylation by inhibiting the ATP binding site within the cell of the EGFR [100].

The Cancer Institutes of Canada are conducting phase II and III investigations, erlotinib treatment resulted in a 12 percent reduction in lung cancer symptoms in NSCLC patients [100]. In phase III/IV clinical studies, 150 mg/kg erlotinib significantly reduced lung cancer metastasis and resulted in three and eight months survival without progression and overall survival, respectively [95]. Despite its high efficacy, erlotinib treatment was difficult to maintain because to its numerous negative side effects, especially on the skin, gut, and eyes [95]. As a result, lower doses and erlotinib therapy interruptions have been proposed as ways to manage these adverse effects [95]. Patients who had never smoked, on the other hand, had an excellent overall survival rate and did not develop [101].

Erlotinib have specific in the 2^{nd} and 3^{rd} line therapy for NSCLC & SCLC patients, according to clinical trials [102]. As a result, In lung cancer patients, trials with 150 mg/kg erlotinib were conducted who had already had chemotherapy with platinum showed significantly when compared to erlotinib alone, there was an improvement in overall survival and progression-free survival [95]. When compared to single first-line chemotherapy, erlotinib co-treatment enhanced NSCLC patients' survival rates [103]. Erlotinib proved very effective as a second-line treatment, especially in patients with advanced NSCLC [104,105]. Combination therapy significantly improved the patients' quality of life by reducing cough, respiratory discomfort, and chest pain and discomfort [106]. Patients demonstrated 60-70 percent recovery and considerable increases in physical abilities after therapy, with the top three symptoms falling between 35 and 45 percent of placebo [106]. Erlotinib exhibited few side effects as a second or third-line treatment, with diarrhoea and minor skin irritations being the most common [95]. In combination with docetaxel and pemetrexed chemotherapeutics, Erlotinib are very effective as a second and third-line treatment [107]. Indeed, erlotinib has appeard as most effective 3^{rd} line treatment to patients that have impaired performance status, whose prospects of survival and quality of life have significantly reduced [107]. As a third-line treatment for lung cancer, erlotinib improved not only quality of life but including the symptoms of palliative care [108,109], and the medicine was well tolerated [110]. When compared to chemotherapeutics, erlotinib co-treatment had a significant cost advantage [102].

B. GEFITINIB - Using data from two sources randomised Iressa Dose Evaluation in Advanced Lung Cancer-1 (IDEAL-1) and IDEAL-2 are phase II trials [82]. Food and Drug Administration

(FDA) approved gefitinib as a 3rd line treatment in 2003. In the IDEAL-1 research, a dose of 250 mg/kg gefitinib enhanced In patients who had previously chemotherapy, the response rate, clinical recovery, overall survival, and progression-free survival were all measured [82,93]. Most crucially, the IDEAL-1 research found that gefitinib had fewer negative effects [93]. Patients with NSCLC who had already received two chemotherapy regimens were evaluated with gefibitinb in the IDEAL-2 clinical trials [82]. Despite the failure of platinum-based and docetaxel chemotherapies, the medication avoided metastasis and enhanced response rates [111]. However, in a trial of phase III with 1700 patients, 250 mg/kg of gefitinib paired to best supportive care did not enhance overall survival [112]. These studies aided the FDA's decision to require the use of gefitinib in patients who had previously had chemotherapeutic treatment [112,102]. In a trial of Phase III inside lung cancer patients that have mild to moderate metastases in the United States, the effects of a well-known lung cancer chemotherapy, docetaxel, and gefitinib were essentially identical according to median overall survival and quality of life [113]. Similarly, the effects of two medicines were nearly identical in patients who were not smokers [113]. Patients with increased EGFR gene expression and a history of smoking, on the other hand, responded better to gefitinib [113]. These findings suggest that gefitinib outperforms EGFR-dependent lung cancer cell growth and mitosis [113]. In comparison to docetaxel, gefitinib was well tolerated and had fewer adverse effects [113].

Despite the fact that erlotinib and gefitinib was only medications that a track record as 2nd and 3rd line therapies for lung cancer, the particular first-generation EGFR tyrosine kinase inhibitors have several drawbacks [114]. The most striking finding was In individuals who have developed resistance to erlotinib or gefitinib, had been on the treatments for a long time [115-116]. Patients with a secondary missense mutation in exon 10 in regards to the EGFR-tyrosine kinase gene, known as the T790M "gate-keeper mutation," were more resistant towards erlotinib & gefitinib [117,118]. In fact, T790M mutation in the EGFR gene was found in 50-60% of patients resistant to erlotinib & gefitinib [117,118]. Furthermore, lung cancer was resistant to erlotinib and gefitinib due to increased hepatocyte growth factor or non-EGFR growth factors [119,120]. To avoid these issues, afatinib, It was proposed to develop a second-generation irreversible tyrosine kinase inhibitor for lung cancer treatment [114].

C. AFATINIB - Afatinib acts by interacting to cysteine 773, 805, and 803 residues in EGFR-tyrokine kinases, it acts as an EGFR inhibitor, especially ErbB4 [121,122]. Afatinib suppresses EGFR and ErbB4 dimerization as well as HER2 heteromerization [123]. Afatinib was found to be successful in lung cancer patients who had previously failed to respond to erlotinib or gefitinib, as well as cell lines from lung cancer with HER2 and T790M mutations in the EGFR gene [121,124]. Afatinib's oral dosage effectiveness of roughly 50 mg/day was demonstrated in a few phase I clinical investigations [125,126]. Patients given 50 mg/day afatinib developed skin rashes and stomach problems, therefore a dose of 40 mg/day was Selected as advanced lung cancer clinical trials [123,127]. This dose exhibited practically identical efficacies (as 50 mg/Kg) and had less side effects. A phase I clinical trial with 40 mg/day afatinib and 250 mg/m2 cetaximib, follows phase II trial along 500 mg/m2 cetaximib, showed a 30 percent overall response and a 75 percent partial response among 97 NSCLC patients [128]. Both with and without T790M mutations, the medication combinations showed 30-36 percent partial response [114]. Patients with the T790M secondary mutation showed roughly 50% stability compared to 30% in individuals without the mutation, showing that afatinib is more successful in patients with the mutation [128]. Similarly, afatinib was found to have a 60 percent objective response rate in 129 patients with EGFR mutations at Phase II of clinical trial (LUX-Lung 2 study) [127]. Nonetheless, research on these afatinib combination therapies is ongoing, and the in the combination therapy technique, the preventive dose and duration of each drugs have yet to be standardised [114].

The Phase III LUX-Lung-5 trial found that combining afatinib with chemotherapeutics and tyrosine kinase inhibitors was helpful [84]. Patients in this study were given 40-50 mg 12 weeks with afatinib orally before being given a combination with paclitaxel [123]. When compared to solo therapy, the combination treatments resulted in better progression-free survival for six months and a significantly higher overall response rate [123]. Afitinib and cetuximab co-treatment was successful in NSCLC patients who had failed to respond to erlotinib & gefitinib plus tyrosine kinase singleclone antibody (cetuximab) [121]. The combination of erlotinib/gefitinib and cetuximab was not effective because of the patients' EGFR T790M mutation, which may be addressed by afatinib and cetuximab [129]. A preliminary study investigation NSCLC mice transgenic for EGFR T790M found that afatinib and cetuximab combined therapies resulted in significant recovery [121]. Afatinib's progression-free survival

was four months longer than cisplatin and pemetrexed or cisplatin as well as gemcitabine, platinum-based double-used chemotherapies, in relation to the LUX-Lung 3 & LUX-Lung 6 phase III studies [130]. Patients with a L858R mutation or exon-19 deletion in the EGFR gene recovered more quickly with afatinib [130]. A research found that 630 of 700 patients treated with afatinib, takes three-month longer continuity than standard chemotherapies (27 months against 24 months, respectively) [123,131]. Furthermore, 699 patients with squamous lung cancer that doesnot respond to platinum-based chemotherapies were studied in a Phase III clinical trial found that afatinib treatment resulted in a five-month longer median progression-free survival than erlotinib treatment [131]. Although the overall response rate for both afatinib and erlotinib-treated patients was nearly comparable, the disease control rate for the former was significantly better [131]. Despite these positive results, afatanib's side effects were sometimes excruciating, including reports of severe diarrhoea and mouth ulcers [114].

Afatinib 40 mg daily oral dose has been approved by the FDA like powerful therapy for NSCLC into the patients with EGFR mutations on exon 19 and 21 (L858R) [114]. However, afatinib consist potential to cause resistance in lung cancer patients [114]. EGFR T790M alleles have been found to cause afatinib resistance in lung cancers in vitro [132]. Second, afatinib failed to target mutations in the hepatocyte growth factor receptor, MET, ErbB2, and other genes [121]. As a result, component of afitinib resistance requires more research, both for individual and combination therapy.

D.ICOTINIB - The central nervous system (CNS) metastases has become a common concern in conjunction with lung cancer, both of which have very unfavourable prognoses [133]. Approximately 25% of patients with NSCLC developed brain metastases throughout the diagnosis phase, 50% throughout therapy [133]. Patients with both lung and brain metastases lived for about six months, but those who were left untreated only lived for some weeks [134]. Chemotherapeutic medications about NSCLC ineffecetive to breach blood-brain barrier, with erlotinib, gefitinib, and afatinib reaching only as far as extracranial lesions [135]. Icotinib, an EGFR-TK inhibitor marketed in China under Conmana was the first brand name medicine demonstrated that efficacious for both NSCLC and brain metastases [136]. As a result, icotinib was deemed a novel therapeutic option for patients with advanced NSCLC who had already been treated [136]. Conmona was found to be effective against both NSCLC and CNS metastases in a

phase II clinical trial, and trial of phase 3(ICOGEN) confirmed its efficacy and protection [136]. Response rates were over 80%, overall survival was around 7 months, and progression-free survival was around 15 months [137]. These patients had never smoked and had never had chemotherapeutic treatment [137]. Only a few individuals received radiation before or during their icotinib treatment [137]. Icotinib was also found to stop lung and brain metastases in EGFR-mutated patients in another phase II trial [138,139]. These icotinib-treated patients had a higher response rate and longer survival than those with wild-type EGFR [138,139]. In a phase II of clinical trial in China, icotinib had combined radiation therapy for the entire brain that proved to be effective in treating NSCLC patients with EGFR mutations and CNS metastases [137]. The median progression that free survival for the co-treatment was twelve months compared to eight months for icotinib alone [137,140]. Apart from a few cases of acneiform lesions and diarrhoea, icotinib's adverse effects were milder and did not cause liver damage [140]. Patients who were evaluated for icotinib safety had a disease control rate of roughly 96 percent, with a 21-month overaly life rate and 11-month progression-free life rate [140]. Icotinib was found to be safe for advanced NSCLC patients as well as effective in treating lung cancer and its associated brain metastases [140]. Despite this, icotinib has less laboratory and clinical trials than gefitinib and erlotinib [140]. As a result, more global multi-centric trials are needed to offer perfect evidence on icotinib's effectiveness and safety [140].

5. SELECTIVE USAGE OF 2ND AND 3RD LINE TREATMENT

In general, 1st and 2nd line therapeutic strategies are commonly employed at lung cancer clinical trials [102]. The approach of 3rd line treatment especially useful for critically ill patients with extensive lung metastases [141]. Patients who actually require 3rd line treatment, on the other hand, are difficult to detect [102]. Grade of EGFR mutation, location, age of patient, history of smoking,loss in weight, or amount of tumour must all be carefully considered for these treatments [141]. A single study comparing the effectiveness of erlotinib being 1st, 2nd, and 3rd line treatment found that approx 27%, 45 percent, 28% of patients recovered [142]. At the Princess Margaret Hospital in Toronto, docetaxel was tested as a 2nd and 3rd line treatment to 74 NSCLC clinical cases [107]. A research comparing that patients are treated with docetaxol & tyrosine kinase inhibitors behaves like 2nd & 3rd line therapies found that overall survival and

progression-free survival were nearly identical [143]. Erlotinib, on the other hand, appeared to be a better 2nd and 3rd line therapy, particularly into the patients who had breakdown that react to chemotherapeutic therapies [144]. However, a phase III open-label trial (Focus) and a clinical trial with 477 patients who were given erlotinib or gefitinibon the point of a 2nd line or 3rd line treatment showed that the two medicines had nearly identical overall and progression-free survival rates [144]. As a result, data upon EGFR inhibitors as 2nd and 3rd line treatments, showing comparable efficacies for both, is difficult to come by. To determine individual treatment efficacies, extensive cohort studies comparing the efficacy to 2nd and 3rd line treatments for lung cancer are required.

6. DOCKING

6.1. Ligand structure preparation

The 3D structures of Erlotinib (PubChem CID: 176870) were downloaded from the PubChemdatabase(https://pubchem.ncbi.nlm.nih.gov). It is saved in sdf format and were converted to .pdb format using open babel software. Further, ligand structures were saved in PDBQT file format, using AutoDockTools(ADT)version 1.5.6.

6.2. Protein preparation

The 3D protein structure file of Epidermal Gowth Factor Receptor (PDB ID: 5UGB) was obtained from RCSB protein data Bank. ADT version 1.5.6 was used to delete the water molecules and merged the non-polar hydrogen atoms to the protein structure. The structure was saved in PDBQT file format, for input into AutoDock Vina.

7. DOCKING RESULT

A grid box with dimension of 126 x 116 x 96 $Å^3$ and was centered on 135.200 24.179 58.599(x.y.z value) created and covers the egfr with grind box to do blind docking using ADT tools. Config.txt file was prepared and then ligand and protein information added in the file. Further docking was performed using AutoDockVina. Out of 9 poses of the ligands obtained in

AutoDockVina, the best pose with low free binding energy is 7.5 and present within the binding pocket of protein were selected. The hydrogen bond interactions were identified between the docked complex of ligand and proteins.Hydrophobic Interaction and Hydrogen bond was calculated by Protein Ligand Interaction Profiler(PILIP).

Url-https://plip-tool.biotec.tu-dresden.de/plip-web/plip/result/3455e37c-6beb-49db-8701-2c4e58a412e4

7.1. Hydrophobic Interaction of ligand and protein

Index	Residue	AA	Distance	Ligand atom	Protein atom
1	694A	LEU	3.63	2476	202
2	719A	ALA	3.72	2483	380
3	721	LYS	3.36	2485	393
4	820A	LEU	3.74	2479	1161

Table 2: Hydrophobic interaction of ligand and protein

7.2. Hydrogen bond

Index	Residue	AA	Distance H-A	Distance D-A	Donor angle	Protein donor?	Side chain	Donor atom	Acceptor atom
1	MET	2.54	3.45	3.45	152.59	yes	x	733 [Nam]	2468 [Nar]

Table 3: Hydrogen Bond

8. IMAGES OF DOCKED LIGAND AND PROTEIN

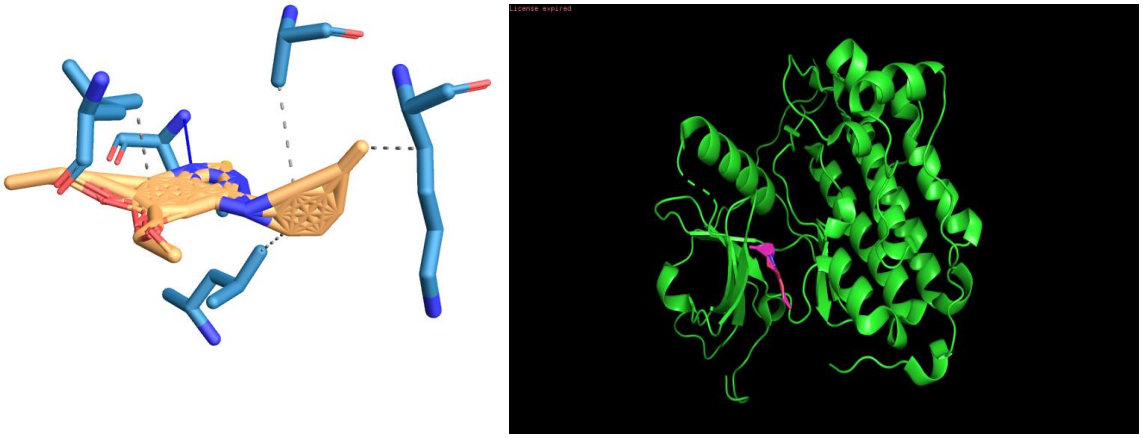

Fig. 3: Ligand and protein interaction

9. CONCLUSION

The epidermal growth factor receptor is a transmembrane protein or tyrosine kinase receptor, we took kinase domain of egfr in docking. It is a clinically validated and have variety of inhibitors, Erlotinib (one of the best inhibitor) as potential drugs for the treatment of Lung Cancer. In our study we tried to use inhibitor and we performed *in silico* docking of the inhibitor We found that the inhibitor showed H-binding interactions with low free binding energy of 7.5. The computationaldata supports the efficacy of erlotinib inhibitor and could be considered as a potent drug against Lung Cancer.

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1. K. Aizawa, C. Liu, S. Tang, *et al.*Tobacco carcinogen induces both lung cancer and non-alcoholic steatohepatitis and hepatocellular carcinomas in ferrets which can be attenuated by lycopene supplementationInt J Cancer, 139 (2016), pp. 1171-1181

2. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factorrelated peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19:183-232.

3. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol 2006;7:505-16.

4. Hynes NH, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005;5:341-54.

5. Sporn MB, Todaro GJ. Autocrine secretion and malignant transformation of cells. N Engl J Med 1980;303:878-80

6. Schlessinger J (2014) Receptor tyrosine kinases: legacy of the first two decades. Cold Spring Harb perspect Biol 6, a008912

7. Yarden Y and Pines G (2012) The ERBB network: at last, cancer therapy meets systems biology. Nat Rev Cancer 12, 553–563.

8. Lemmon MA and Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141, 1117–1134

9. Tan X, Lambert PF, Rapraeger AC and Anderson RA (2016a) Stress-induced EGFR trafficking: mechanisms, functions, and therapeutic implications. Trends Cell Biol 26, 352–366

10. Jutten B, Keulers TG, Schaaf MB, Savelkouls K, Theys J, Span PN, Vooijs MA, Bussink J and Rouschop KM (2013) EGFR overexpressing cells and tumors are dependent on autophagy for growth and survival. Radiother Oncol 108, 479–483.

11. Zhang X, Gureasko J, Shen K, Cole PA and Kuriyan J (2006) An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. Cell 125, 1137–1149

12. Cairns RA, Harris IS and Mak TW (2011) Regulation of cancer cell metabolism. Nat Rev Cancer 11, 85–95

13. Guo D, Prins RM, Dang J, Kuga D, Iwanami A, Soto H, Lin KY, Huang TT, Akhavan D, Hock MB et al. (2009) EGFR signaling through an Akt-SREBP-1- dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy. Sci Signal 2, ra82

14. Makinoshima H, Takita M, Matsumoto S, Yagishita A, Owada S, Esumi H and Tsuchihara K (2014) Epidermal growth factor receptor (EGFR) signaling regulates global metabolic pathways in EGFRmutated lung adenocarcinoma. J Biol Chem 289, 20813–20823.

15. Lim SO, Li CW, Xia W, Lee HH, Chang SS, Shen J, Hsu JL, Raftery D, Djukovic D, Gu H et al. (2016) EGFR signaling enhances aerobic glycolysis in triple-negative breast cancer cells to promote tumor growth and immune escape. Cancer Res 76, 1284–1296.

16. Zhang J, Song F, Zhao X, Jiang H, Wu X, Wang B, Zhou M, Tian M, Shi B, Wang H et al. (2017) EGFR modulates monounsaturated fatty acid synthesis through phosphorylation of SCD1 in lung cancer. Mol Cancer 16, 127

17. Babic I, Anderson ES, Tanaka K, Guo D, Masui K, Li B, Zhu S, Gu Y, Villa GR, Akhavan D et al. (2013) EGFR mutation-induced alternative splicing of Max contributes to growth of glycolytic tumors in brain cancer. Cell Metab 17, 1000–1008.

18. Makinoshima H, Takita M, Saruwatari K, Umemura S, Obata Y, Ishii G, Matsumoto S, Sugiyama E, Ochiai A, Abe R et al. (2015) Signaling through the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) axis is responsible for aerobic glycolysis mediated by glucose transporter in epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma. J Biol Chem 290, 17495–17504.

19. DeBerardinis RJ and Chandel NS (2016) Fundamentals of cancer metabolism. Sci Adv 2, e1600200

20. Masui K, Cavenee WK and Mischel PS (2014) mTORC2 in the center of cancer metabolic reprogramming. Trends Endocrinol Metab 25, 364–373.

21. Guo D, Reinitz F, Youssef M, Hong C, Nathanson D, Akhavan D, Kuga D, Amzajerdi AN, Soto H, Zhu S et al. (2011) An LXR agonist promotes glioblastoma cell death through inhibition of an EGFR/AKT/ SREBP-1/LDLR-dependent pathway. Cancer Discov 1, 442–456.

22. Barron CC, Bilan PJ, Tsakiridis T and Tsiani E (2016) Facilitative glucose transporters: implications for cancer detection, prognosis and treatment. Metabolism 65, 124–139.

23. Weihua Z, Tsan R, Huang WC, Wu Q, Chiu CH, Fidler IJ and Hung MC (2008) Survival of cancer cells is maintained by EGFR independent of its kinase activity. Cancer Cell 13, 385–393

24. Wieman HL, Wofford JA and Rathmell JC (2007) Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. Mol Biol Cell 18, 1437–1446

25. Wofford JA, Wieman HL, Jacobs SR, Zhao Y and Rathmell JC (2008) IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. Blood 111, 2101– 2111

26. Hong SY, Yu FX, Luo Y and Hagen T (2016) Oncogenic activation of the PI3K/Akt pathway promotes cellular glucose uptake by downregulating the expression of thioredoxin-interacting protein. Cell Signal 28, 377– 383.

27. Waldhart AN, Dykstra H, Peck AS, Boguslawski EA, Madaj ZB, Wen J, Veldkamp K, Hollowell M, Zheng B, Cantley LC et al. (2017) Phosphorylation of TXNIP by AKT mediates acute influx of glucose in response to insulin. Cell Rep 19, 2005–2013.

28. Yarden Y, Sliwkowski M: Untangling the ErbB signaling network. Nat Rev Mol Cell Biol 2:127-137, 2001.

29. Kuan CT, Wikstrand CJ, Bigner DD: EGF mutant receptor vIII as a molecular target in cancer therapy. Endocr Relat Cancer 8:83-96, 2001.

30. Prenzel N, Zwick E, Daub H, et al: EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHBEGF. Nature 402:884-888, 1999.

31. Liu D, Ghiso JA, Estrada Y, et al: EGFR is a transducer of the urokinase receptor initiated signal that is required for in vivo growth of a human carcinoma. Cancer Cell 1:445-457, 2002.

32. Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 103:211-225, 2000.

33.Alroy I, Yarden Y: The ErbB signaling network in embryogenesis and oncogenesis: Signal diversification through combinatorial ligand-receptor interactions. FEBS Lett 410:83-86, 1997.

34. Lewis TS, Shapiro PS, Ahn NG: Signal transduction through MAP kinase cascades. Adv Cancer Res 74:49-139, 1998.

35. Chan TO, Rittenhouse SE, Tsichlis PN: AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositidedependent phosphorylation. Annu Rev Biochem 68:965-1014, 1999.

36. Vivanco I, Sawyers CL: The phosphatidylinositol 3-Kinase-Akt pathway in human cancer. Nat Rev Cancer 2:489-501, 2002.

37. Kawamoto T, Sato JD, Le A, et al: Growth stimulation of A431 cells by EGF: Identification of high affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc Natl Acad SciUSA 80:1337-1341, 1983.

38. Masui H, Kawamoto T, Sato JD, et al: Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res 44:1002-1007, 1984.

39. Mendelsohn J: Targeting the epidermal growth factor receptor for cancer therapy. J Clin Oncol 20:1s-13s, 2002 (suppl 18).

40. Salomon D, Brandt R, Ciardiello F, et al: Epidermal growth factorrelated peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 19:183-232, 1995.

41. Moscatello DK: Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. Cancer Res 55:5536-5539, 1995.

42. Nishikawa R: A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad SciUSA 91:7727-7731, 1994.

43. Grandis JR, Melhem MF, Gooding WE, et al: Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst 90:824-832, 1998.

44. Rusch V, Baselga J, Cordon-Cardo C, et al: Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res 53:2379-2385, 1993.

45. Hynes NH, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 200.

46. Normanno N, Bianco C, De Luca A, Maiello MR, Salomon DS. Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment. Endocr Relat Cancer 2003;10:1-21.

47. Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res 2001;7:2958-70.

48. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 2003;21:2787-99.

49. Li S, Schmitz KR, Jeffrey PD, Wiltzius JJW, Kussie P, Ferguson KM. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 2005;7:301-11.

50. Mukohara T, Engelman JA, HannaNH, et al. Differential effects of gefitinib and cetuximab on non-small-cell lung cancers bearing epidermal growth factor receptor mutations. J Natl Cancer Inst 2005;97:1185-94.

51. Imai K, Takaoka A. Comparing antibody and small-molecule therapies for cancer. Nat Rev Cancer 2006;6:714-27.

52. Fong KM, Sekido Y, Minna JD. Molecular pathogenesis of lung cancer. *J Thorac Cardiovasc Surg.* 1999;118:1136–1152.

53. Carcereny E, Moran T, Capdevila L, Cros S, Vila L, de Los Llanos Gil M, Remon J, Rosell R. The epidermal growth factor receptor (EGRF) in lung cancer. *Transl Respir Med.* 2015;3:1.

54. Woll PJ. New perspectives in lung cancer. 2. Growth factors and lung cancer. *Thorax.* 1991;46:924–929.

55. Ciardiello F, Tortora G. Interactions between the epidermal growth factor receptor and type I protein kinase a: biological significance and therapeutic implications. *Clin Cancer Res.* 1998;4:821–828.

56. Marmor MD, Skaria KB, Yarden Y. Signal transduction and oncogenesis by ErbB/HER receptors. *Int J Radiat Oncol Biol Phys.* 2004;58:903–913.

57. Zandi R, Larsen AB, Andersen P, Stockhausen MT, Poulsen HS. Mechanisms for oncogenic activation of the epidermal growth factor receptor. *Cell Signal.* 2007;19:2013–2023.

58. Hodkinson PS, Mackinnon A, Sethi T. Targeting growth factors in lung cancer. *Chest.* 2008;133:1209–1216.

59. Tanaka T, Matsuoka M, Sutani A, Gemma A, Maemondo M, Inoue A, Okinaga S, Nagashima M, Oizumi S, Uematsu K, Nagai Y, Moriyama G, Miyazawa H, Ikebuchi K, Morita S, Kobayashi K, Hagiwara K. Frequency of and variables associated with the EGFR mutation and its subtypes. *Int J Cancer.* 2010;126:651–655.

60. Gahr S, Stoehr R, Geissinger E, Ficker JH, Brueckl WM, Gschwendtner A, Gattenloehner S, Fuchs FS, Schulz C, Rieker RJ, Hartmann A, Ruemmele P, Dietmaier W. EGFR mutational status in a large series of caucasian european NSCLC patients: data from daily practice. *Br J Cancer.* 2013;109:1821–1828.

61. Sakuma Y, Matsukuma S, Yoshihara M, Nakamura Y, Noda K, Nakayama H, Kameda Y, Tsuchiya E, Miyagi Y. Distinctive evaluation of nonmucinous and mucinous subtypes of bronchioloalveolar carcinomas in EGFR and K-ras gene-mutation analyses for Japanese lung adenocarcinomas: confirmation of the correlations with histologic subtypes and gene mutations. *Am J Clin Pathol.* 2007;128:100–108.

62.Tam IY, Chung LP, Suen WS, Wang E, Wong MC, Ho KK, Lam WK, Chiu SW, Girard L, Minna JD, Gazdar AF, Wong MP. Distinct epidermal growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features. *Clin Cancer Res.* 2006;12:1647–1653.

63. Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, Camplese PP, Iarussi T, Mucilli F, Mezzetti A, Cuccurullo F, Sacco R, Buttitta F. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J. Clin. Oncol.* 2005;23:857–865.

64. oshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, Kawakami F, Tsuruyama T, Travis WD, Date H, Haga H. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. *J Thorac Oncol.* 2013;8:52–61.

65. Prabhakar CN. Epidermal growth factor receptor in non-small cell lung cancer. *Transl Lung Cancer Res.* 2015;4:110–118.

66. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med.* 2005;353:172–187.

67. Lee DC, Fenton SE, Berkowitz EA, Hissong MA. Transforming growth factor alpha: expression, regulation, and biological activities. *Pharmacol Rev.* 1995;47:51–85.

68. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell.* 2010;141:1117–1134.

69. Yarden Y. The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities. *Eur J Cancer.* 2001;37(Suppl 4):S3–8.

70. Cohen S, Carpenter G, King L Jr. Epidermal growth factor-receptor-protein kinase interactions. *Prog Clin Biol Res.* 1981;66:557–567.

71. Siegelin MD, Borczuk AC. Epidermal growth factor receptor mutations in lung adenocarcinoma. *Lab Invest.* 2014;94:129–137.

72. Busser B, Sancey L, Josserand V, Niang C, Khochbin S, Favrot MC, Coll JL, Hurbin A. Amphiregulin promotes resistance to gefitinib in nonsmall cell lung cancer cells by regulating Ku70 acetylation. *Mol Ther.* 2010;18:536–543.

73. Tsao MS, Zhu H, Viallet J. Autocrine growth loop of the epidermal growth factor receptor in normal and immortalized human bronchial epithelial cells. *Exp Cell Res.* 1996;223:268–273.

74. Shoelson SE. SH2 and PTB domain interactions in tyrosine kinase signal transduction. *Curr Opin Chem Biol.* 1997;1:227–234.

75. Cox G, Steward WP, O'Byrne KJ. The plasmin cascade and matrix metalloproteinases in non-small cell lung cancer. *Thorax.* 1999;54:169–179.

76. Cox G, Walker RA, Andi A, Steward WP, O'Byrne KJ. Prognostic significance of platelet and microvessel counts in operable non-small cell lung cancer. *Lung Cancer.* 2000;29:169–177.

77. Giatromanolaki A, Koukourakis M, O'Byrne K, Fox S, Whitehouse R, Talbot DC, Harris AL, Gatter KC. Prognostic value of angiogenesis in operable non-small cell lung cancer. *J Pathol.* 1996;179:80–88.

78. Bruns CJ, Solorzano CC, Harbison MT, Ozawa S, Tsan R, Fan D, Abbruzzese J, Traxler P, Buchdunger E, Radinsky R, Fidler IJ. Blockade of the epidermal growth factor receptor signaling by a novel tyrosine kinase inhibitor leads to apoptosis of endothelial cells and therapy of human pancreatic carcinoma. *Cancer Res.* 2000;60:2926–2935.

79. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer Res.* 1999;59:1935–1940.

80. Perrotte P, Matsumoto T, Inoue K, Kuniyasu H, Eve BY, Hicklin DJ, Radinsky R, Dinney CP. Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin Cancer Res.* 1999;5:257–265.

81. Langer CJ. Emerging role of epidermal growth factor receptor inhibition in therapy for advanced malignancy: focus on NSCLC. *Int J Radiat Oncol Biol Phys.* 2004;58:991–1002.

82. Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis H, Sandler A, Albain KS, Cella D, Wolf MK, Averbuch SD, Ochs JJ, Kay AC. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in

symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA.* 2003;290:2149–2158.

83. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, Langer CJ, DeVore RF 3rd, Gaudreault J, Damico LA, Holmgren E, Kabbinavar F. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J. Clin. Oncol.* 2004;22:2184–2191.

84. Raben D, Helfrich B, Chan DC, Ciardiello F, Zhao L, Franklin W, Baron AE, Zeng C, Johnson TK, Bunn PA Jr. The effects of cetuximab alone and in combination with radiation and/or chemotherapy in lung cancer. *Clin Cancer Res.* 2005;11:795–805.

85. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science.* 2004;304:1497–1500.

86. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst.* 2005;97:339–346.

87. Hanna N, Lilenbaum R, Ansari R, Lynch T, Govindan R, Janne PA, Bonomi P. Phase II trial of cetuximab in patients with previously treated non-small-cell lung cancer. *J. Clin. Oncol.* 2006;24:5253–5258.

88. Robert F, Blumenschein G, Herbst RS, Fossella FV, Tseng J, Saleh MN, Needle M. Phase I/IIa study of cetuximab with gemcitabine plus carboplatin in patients with chemotherapy-naive advanced non-small-cell lung cancer. *J. Clin. Oncol.* 2005;23:9089–9096.

89. Thienelt CD, Bunn PA Jr, Hanna N, Rosenberg A, Needle MN, Long ME, Gustafson DL, Kelly K. Multicenter phase I/II study of cetuximab with paclitaxel and carboplatin in untreated patients with stage IV non-small-cell lung cancer. *J. Clin. Oncol.* 2005;23:8786–8793.

90. Rosell R, Robinet G, Szczesna A, Ramlau R, Constenla M, Mennecier BC, Pfeifer W, O'Byrne KJ, Welte T, Kolb R, Pirker R, Chemaissani A, Perol M, Ranson MR, Ellis PA, Pilz K, Reck M. Randomized phase II study of cetuximab plus cisplatin/vinorelbine compared with cisplatin/vinorelbine alone as first-line therapy in EGFR-expressing advanced non-small-cell lung cancer. Ann Oncol. 2008;19:362–369.

91. Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, Eckhardt SG, Tolcher A, Britten CD, Denis L, Ferrante K, Von Hoff DD, Silberman S, Rowinsky EK. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J. Clin. Oncol. 2001;19:3267–3279.

92. Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, Rojo F, Hong WK, Swaisland H, Averbuch SD, Ochs J, LoRusso PM. Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. *J. Clin. Oncol.* 2002;20:3815–3825.

93. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong RP, Baselga J. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected] . *J. Clin. Oncol.* 2003;21:2237–2246.

94. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (iressa survival evaluation in lung cancer) *Lancet.* 2005;366:1527–1537.

95. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabárbara P, Seymour L National Cancer Institute of Canada Clinical Trials Group. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med.* 2005;353:123–132.

96. Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, Ichimura K, Tsuda T, Yano M, Tsukuda K, Tabata M, Ueoka H, Tanimoto M, Date H, Gazdar AF, Shimizu N. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer *Res.* 2005;11:1167–1173.

97. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med.* 2005;352:786–792.

98. Kobayashi S, Ji H, Yuza Y, Meyerson M, Wong KK, Tenen DG, Halmos B. An alternative inhibitor overcomes resistance caused by a mutation of the epidermal growth factor receptor. *Cancer Res.* 2005;65:7096–7101.

99. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci *U S A.* 2004;101:13306–13311.

100. Perez-Soler R, Chachoua A, Hammond LA, Rowinsky EK, Huberman M, Karp D, Rigas J, Clark GM, Santabarbara P, Bonomi P. Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. J. Clin. Oncol. 2004;22:3238–3247.

101. Clark GM, Zborowski DM, Santabarbara P, Ding K, Whitehead M, Seymour L, Shepherd FA National Cancer Institute of Canada Clinical Trials Group. Smoking history and epidermal growth factor receptor expression as predictors of survival benefit from erlotinib for patients with non-small-cell lung cancer in the national cancer institute of canada clinical trials group study BR. 21. Clin Lung Cancer. 2006;7:389–394.

102. Syrigos KN, Saif MW, Karapanagiotou EM, Oikonomopoulos G, De Marinis F. The need for third-line treatment in non-small cell lung cancer: an overview of new options. Anticancer Res. 2011;31:649–659.

103. Hensing TA, Schell MJ, Lee JH, Socinski MA. Factors associated with the likelihood of receiving second line therapy for advanced non-small cell lung cancer. *Lung Cancer.* 2005;47:253–259.

104. Rossi D, Dennetta D, Ugolini M, Catalano V, Alessandroni P, Giordani P, Baldelli AM, Casadei V, Graziano F, Luzi Fedeli S. Activity and safety of erlotinib as second-and third-line treatment in elderly patients with advanced non-small cell lung cancer: a phase II trial. *Target Oncol.* 2010;5:231–235.

105. Lyseng-Williamson KA. Erlotinib: a pharmacoeconomic review of its use in advanced non-small cell lung cancer. *Pharmacoeconomics.* 2010;28:75–92.

106. Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, Liu N, Zhang T, Marrano P, Whitehead M, Squire JA, Kamel-Reid S, Seymour L, Shepherd FA, Tsao MS National Cancer Institute of Canada Clinical Trials Group Study BR.21. Symptom improvement in lung cancer patients treated with erlotinib: quality of life analysis of the national cancer institute of canada clinical trials group study BR.21. *J. Clin. Oncol.* 2006;24:3831–3837.

107. Ng R, Loreto M, Lee R, Leighl NB. Brief report: retrospective review of efficacy of erlotinib or gefitinib compared to docetaxel as subsequent line therapy in advanced non-small cell lung cancer (NSCLC) following failure of platinum-based chemotherapy. *Lung Cancer.* 2008;61:262–265.

108. Dancey J, Shepherd FA, Gralla RJ, Kim YS. Quality of life assessment of second-line docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy: results of a prospective, randomized phase III trial. *Lung Cancer.* 2004;43:183–194.

109. De Marinis F, Pereira JR, Fossella F, Perry MC, Reck M, Salzberg M, Jassem J, Peterson P, Liepa AM, Moore P, Gralla RJ. Lung cancer symptom scale outcomes in relation to standard efficacy measures: an analysis of the phase III study of pemetrexed versus docetaxel in advanced non-small cell lung cancer. *J Thorac Oncol.* 2008;3:30–36.

110. Cappuzzo F, Ciuleanu T, Stelmakh L, Cicenas S, Szczesna A, Juhasz E, Esteban E, Molinier O, Brugger W, Melezinek I, Klingelschmitt G, Klughammer B, Giaccone G SATURN

investigators. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. *Lancet Oncol.* 2010;11:521–529.

111. Cohen MH, Williams GA, Sridhara R, Chen G, McGuinn WD Jr, Morse D, Abraham S, Rahman A, Liang C, Lostritto R, Baird A, Pazdur R. United states food and drug administration drug approval summary: gefitinib (ZD1839; Iressa) tablets. *Clin Cancer Res.* 2004;10:1212–1218.

112. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (iressa survival evaluation in lung cancer) *Lancet.* 2005;366:1527–1537.

113. Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, Li LY, Watkins CL, Sellers MV, Lowe ES, Sun Y, Liao ML, Osterlind K, Reck M, Armour AA, Shepherd FA, Lippman SM, Douillard JY. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. *Lancet.* 2008;372:1809–1818.

114. Joshi M, Rizvi SM, Belani CP. Afatinib for the treatment of metastatic non-small cell lung cancer. *Cancer Manag Res.* 2015;7:75–82.

115. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med.* 2009;361:947–957.

116. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K, Fukuoka M West Japan Oncology Group. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol.* 2010;11:121–128.

117. Arcila ME, Oxnard GR, Nafa K, Riely GJ, Solomon SB, Zakowski MF, Kris MG, Pao W, Miller VA, Ladanyi M. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. *Clin Cancer Res.* 2011;17:1169–1180.

118. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, Kris MG, Miller VA, Ladanyi M, Riely GJ. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res.* 2013;19:2240–2247.

119. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, Bergethon K, Shaw AT, Gettinger S, Cosper AK, Akhavanfard S, Heist RS, Temel J, Christensen JG, Wain JC, Lynch TJ, Vernovsky K, Mark EJ, Lanuti M, Iafrate AJ, Mino-Kenudson M, Engelman JA. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med.* 2011;3:75ra26.

120. Li T, Kung HJ, Mack PC, Gandara DR. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. *J. Clin. Oncol.* 2013;31:1039–1049.

121. Chen X, Zhu Q, Zhu L, Pei D, Liu Y, Yin Y, Schuler M, Shu Y. Clinical perspective of afatinib in non-small cell lung cancer. *Lung Cancer.* 2013;81:155–161.

122. Solca F, Dahl G, Zoephel A, Bader G, Sanderson M, Klein C, Kraemer O, Himmelsbach F, Haaksma E, Adolf GR. Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. *J Pharmacol Exp Ther.* 2012;343:342–350.

123. Yap TA, Popat S. Toward precision medicine with next-generation EGFR inhibitors in non-small-cell lung cancer. *Pharmgenomics Pers Med.* 2014;7:285–295.

124. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, Padera RF, Shapiro GI, Baum A, Himmelsbach F, Rettig WJ, Meyerson M, Solca F, Greulich H, Wong KK. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. *Oncogene.* 2008;27:4702–4711.

125. Murakami H, Tamura T, Takahashi T, Nokihara H, Naito T, Nakamura Y, Nishio K, Seki Y, Sarashina A, Shahidi M, Yamamoto N. Phase I study of continuous afatinib (BIBW 2992) in patients with advanced non-small cell lung cancer after prior chemotherapy/erlotinib/gefitinib (LUX-Lung 4) Cancer Chemother Pharmacol. 2012;69:891–899.

126. Yap TA, Vidal L, Adam J, Stephens P, Spicer J, Shaw H, Ang J, Temple G, Bell S, Shahidi M, Uttenreuther-Fischer M, Stopfer P, Futreal A, Calvert H, de Bono JS, Plummer R. Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. J. Clin. Oncol. 2010;28:3965–3972.

127. Yang JC, Wu YL, Schuler M, Sebastian M, Popat S, Yamamoto N, Zhou C, Hu CP, O'Byrne K, Feng J, Lu S, Huang Y, Geater SL, Lee KY, Tsai CM, Gorbunova V, Hirsh V, Bennouna J, Orlov S, Mok T, Boyer M, Su WC, Lee KH, Kato T, Massey D, Shahidi M, Zazulina V, Sequist LV. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol. 2015;16:141–151.

128. Ribeiro Gomes J, Cruz MR. Combination of afatinib with cetuximab in patients with EGFR-mutant non-small-cell lung cancer resistant to EGFR inhibitors. *Onco* Targets Ther. 2015;8:1137–1142.

129. Ribeiro Gomes J, Cruz MR. Combination of afatinib with cetuximab in patients with EGFR-mutant non-small-cell lung cancer resistant to EGFR inhibitors. *Onco* Targets Ther. 2015;8:1137–1142.

130. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, Geater SL, Orlov S, Tsai CM, Boyer M, Su WC, Bennouna J, Kato T, Gorbunova V, Lee KH, Shah R, Massey D, Zazulina V, Shahidi M, Schuler M. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J*. Clin. Oncol. 2013;31:3327–3334.

131. Melosky B. Review of EGFR TKIs in metastatic NSCLC, including ongoing trials. *Front Oncol*. 2014;4:244.

132. Kim Y, Ko J, Cui Z, Abolhoda A, Ahn JS, Ou SH, Ahn MJ, Park K. The EGFR T790M mutation in acquired resistance to an irreversible second-generation EGFR inhibitor. *Mol Cancer Ther.* 2012;11:784–791.

133. Villano JL, Durbin EB, Normandeau C, Thakkar JP, Moirangthem V, Davis FG. Incidence of brain metastasis at initial presentation of lung cancer. *Neuro Oncol.* 2015;17:122–128.

134. Langer CJ, Mehta MP. Current management of brain metastases, with a focus on systemic options. *J. Clin. Oncol.* 2005;23:6207–6219.

135. Fekrazad MH, Ravindranathan M, Jones DV Jr. Response of intracranial metastases to erlotinib therapy. *J. Clin. Oncol.* 2007;25:5024–5026.

136. Shi Y, Zhang L, Liu X, Zhou C, Zhang L, Zhang S, Wang D, Li Q, Qin S, Hu C, Zhang Y, Chen J, Cheng Y, Feng J, Zhang H, Song Y, Wu YL, Xu N, Zhou J, Luo R, Bai C, Jin Y, Liu W, Wei Z, Tan F, Wang Y, Ding L, Dai H, Jiao S, Wang J, Liang L, Zhang W, Sun Y. Icotinib versus gefitinib in previously treated advanced non-small-cell lung cancer (ICOGEN): a randomised, double-blind phase 3 non-inferiority trial. *Lancet Oncol.* 2013;14:953–961.

137. Fan Y, Huang Z, Fang L, Miao L, Gong L, Yu H, Yang H, Lei T, Mao W. A phase II study of icotinib and whole-brain radiotherapy in Chinese patients with brain metastases from non-small cell lung cancer. *Cancer Chemother Pharmacol.* 2015;76:517–523.

138. Eichler AF, Kahle KT, Wang DL, Joshi VA, Willers H, Engelman JA, Lynch TJ, Sequist LV. EGFR mutation status and survival after diagnosis of brain metastasis in nonsmall cell lung cancer. Neuro Oncol. 2010;12:1193–1199.

139. Porta R, Sanchez-Torres JM, Paz-Ares L, Massuti B, Reguart N, Mayo C, Lianes P, Queralt C, Guillem V, Salinas P, Catot S, Isla D, Pradas A, Gurpide A, de Castro J, Polo E, Puig T, Taron M, Colomer R, Rosell R. Brain metastases from lung cancer responding to erlotinib: the importance of EGFR mutation. Eur Respir *J.* 2011;37:624–631.

140. Xu J, Liu X, Yang S, Zhang X, Shi Y. Efficacy and safety of icotinib in patients with brain metastases from lung adenocarcinoma. *Onco Targets Ther.* 2016;9:2911–2917.

141. Girard N, Jacoulet P, Gainet M, Elleuch R, Pernet D, Depierre A, Dalphin JC, Westeel V. Third-line chemotherapy in advanced non-small cell lung cancer: identifying the candidates for routine practice. *J Thorac Oncol.* 2009;4:1544–1549.

142. Ailawadhi S, Derby L, Natarajan R, Fetterly G, Reid M, Ramnath N. Erlotinib for metastatic non-small-cell lung cancer: first-, second-or third-line setting-does it matter? A single-institution experience. *Oncology.* 2009;76:85–90.

143. Popat S, Barbachano Y, Ashley S, Norton A, O'Brien M. Erlotinib, docetaxel, and gefitinib in sequential cohorts with relapsed non-small cell lung cancer. *Lung Cancer.* 2008;59:227–231.

144. Ramalingam S, Sandler AB. Salvage therapy for advanced non-small cell lung cancer: factors influencing treatment selection. *Oncologist.* 2006;11:655–665.

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