Chapter 8
Lung Cancer Receptors and Targeting Strategies

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Abstract Lung cancer still remains the leading cause of cancer-related deaths worldwide. Till now, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) have effectively responded to conventional therapy. However, because of cancer nature and subsequent side effects of conventional therapy, inventing novel drug targets for lung cancer therapies has become essential. The disease management recently has seen a paradigm shift with the advent of next-generation sequencing, which has extensively affected the disease prognosis and hence led to newer targeted therapies. Receptors particularly have played an important role as molecular targets and hence presented new opportunities for intracellular targeting of drug delivery systems. Such approach for therapy not only improves the efficacy of the drug but also reduces the overall systemic cytotoxicity. This chapter extensively focuses on such receptors targeted for lung cancer therapy. Further, the role of receptors like epidermal growth factor receptor (EGFR), c-MET, and vascular endothelial growth factor (VEGF) has been discussed with respect to their appropriate ligand(s) binding and developed nanocarrier system for targeting. In addition, this chapter presents the current status of clinical outcomes of conventional drugs in targeting these receptors and thus improving the overall survival rate in patients suffering from this dreaded disease.

Keywords Lung cancer · epidermal growth factor receptor (EGFR) · ligand targeting · c-MET receptor · vascular endothelial growth factor receptor

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Abbreviations

ADC	Adenocarcinomas
ALK	Anaplastic lymphoma kinase
AREG	Amphiregulin
ADCC	Antibody-mediated cellular cytotoxicity
ATP	Adenosine triphosphate
ADAM	A disintegrin and metalloproteinase
BTC	Betacellulin
CRR	Confirmed response rate
EGF	Epidermal growth factor
EPG	Epigen
EPR	Epiregulin
EGFR	Epidermal growth factor receptor
Grb2	Growth factor receptor-bound protein 2
HAP	Hypoxia-activated prodrugs
HIF-1α	Hypoxia inducible factor-1α
HGF	Hepatocyte growth factor
HGFR	Hepatocyte growth factor receptor
HB-EGF	Heparin-binding EGF
mAbs	Monoclonal antibodies
MAPK	Mitogen-activated protein kinase
NSCLC	Non-small cell lung cancer
NRG	Neuregulins
ORR	Objective response rate
PI3K	Phosphatidylinositol 3′-kinase
PLC	Phospholipase C
PLC-γ	Phospholipase C-γ
PEI	Polyethylenimine
RTKs	Receptor tyrosine kinases
SCC	Squamous cell carcinomas
SCLC	Small cell lung cancer
STATs	Signal transducers and activators of transcription
SAR	Structure activity relationship
ScFv	Single chain variable fragment
TGF-α	Transforming growth factor alpha
TKIs	Tyrosine kinase inhibitors
TGFβ1	Transforming growth factor beta 1
TNFα	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

1 Introduction

Lung cancer has been one of the major cancer types that are associated with a high-mortality rate, all over the globe. Lung cancer is more commonly observed in male patients than in females, and has a higher prevalence in the geriatric population [1]. Over the past century, there has been tremendous advancement in the pathophysiological understanding about lung cancer. However, the major drawback associated with this disease is its poor prognosis, often leading to an inoperable condition. Nevertheless, considerable progress is being made in the development of newer strategies against lung cancer, especially with regard to the discovery of newer therapeutic targets, development of various therapeutic molecules, either small molecules or macromolecules like the antibody-based options, and also advanced delivery approaches like the targeted delivery systems or combinatorial therapies.

The molecular basis of lung cancer is complex and heterogeneous. Therefore, it is important to understand the molecular alterations at multiple levels, namely, genetic, epigenetic, and protein expression, and their functional significance, which may have the potential to impact the diagnosis, prognosis, and treatment of lung cancer. Lung cancers may develop through multistep processes involving several genetic and epigenetic alterations, particularly activation of growth-promoting pathways and inhibition of tumor suppressor pathways. A greater understanding of these biochemical pathways is thus crucial for the development of treatment strategies that can target the molecular aberrations underlying lung cancer, as well as their downstream pathways.

1.1 Classification of Lung Cancer

Based on their histology, the lung cancers are classified into two main types, which include the non-small cell lung cancer (NSCLC) and the small cell lung cancer (SCLC). Among these, the NSCLC is more predominant and demonstrates an occurrence of almost 85%, as shown in Fig. 8.1 [2]. The NSCLC is further classified into three major types, namely, the *adenocarcinoma*, the squamous cell carcinoma, and the large-cell carcinoma. The origin of the NSCLC is mostly epithelial, whereas that of the SCLC is neuroendocrine [3, 4]. The main characteristics of the different kinds of lung cancers with respect to their origin, occurrence, and their prominent features have been listed in Table 8.1.

2 Brief Overview of the Receptors Associated with Specific Forms of Lung Cancer

Receptor-mediated tumor targeting has received considerable attention in the field of anticancer therapeutics due to their specific action. Targeting the receptors, overexpressed in cancers, has opened new opportunities for intracellular targeting of

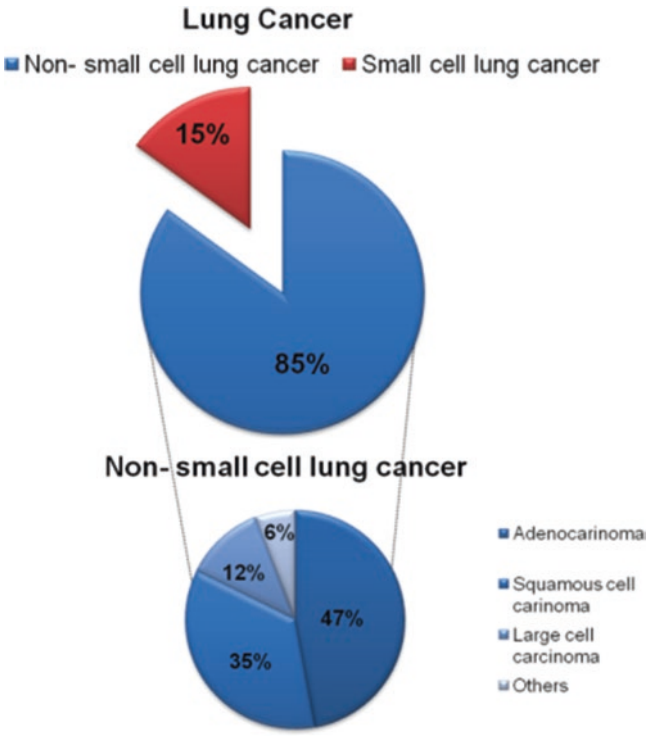


Fig. 8.1 Statistics for the occurrence of different types of lung cancer

drugs and delivery systems that are conjugated with targeting moieties, that is, the ligands. This receptor-mediated targeting of anticancer drugs, especially using nano-sized carrier systems, protects them from the degrading body environment and improves their pharmacokinetic properties by extending their circulation time within the body. Moreover, it also helps to overcome the systemic toxicity and adverse effects that arise due to the nonselective nature of most of the current anticancer therapeutic agents [5].

Recently, a large number of molecular changes, such as mutations and gene amplifications, have been found to be responsible for tumor survival and cancer prognosis [6]. The targeted anticancer therapies also aim to focus on these common cellular modulations that take place at the molecular level. Targeting of these modulations enhances the survival rates in patients, which may not be possible in nonsurgical stages [7]. Personalized therapy can be used to target the cancers, according to the patients' predisposition to them, according to the individuals' genomic profiles, and can hence deliver appropriate drugs, at the correct dose and at the right time [8]. Some of the mediators that may play a predominant role in the treatment of lung cancer include the epidermal growth factor receptor (EGFR), the

Table 8.1 Characteristics of various forms lung cancer

Sr. No	Cancer type	Characteristics
1.	Small cell-lung cancer	Neuroendocrine in origin Highly metastatic and subsequent relapse observed Rarely found in nonsmokers Difficulty in surgical resection
2.	Non-small cell lung cancer	Common form of lung cancer Easily removed by surgical resection, by standard care for localized occurrence
(a) Adenocarcinoma		Major type of NSCLC Caused due to exposure to radiation and carcinogens Originates from peripheral tissue of the lungs, mostly mucus-secreting cells Spreads at a lower rate Bronchioloalveolar carcinoma is adenocarcinoma
(b) Squamous cell carcinoma		Second common type of NSCLC Originates in the airway lining of the lung cells
(c) Large-cell carcinoma		Difficult to treat Originates in the central part of the lungs, may have neuroendocrine origin Quick in growth and spreads rapidly Mostly discovered at later stages

vascular endothelial growth factor (VEGF), the anaplastic lymphoma kinase (ALK), etc. [9]. A comprehensive list of all the prognostic factors or receptors that are significant during the development of lung cancer has been stated in Table 8.2.

Out of these receptors, EGFR, c-MET, and VEGFR have been discussed in details in relevance to lung cancer as they have been extensively studied and exploited for cancer therapy.

3 The EGFR Receptor

The EGFR is highly expressed in almost all types of lung cancers. The receptor has been extensively studied, specifically for targeting the NSCLC, since mutations of EGFR in SCLC patients are rare [36]. This growth factor triggers signaling through the EGFR receptor tyrosine kinase (RTK), which promotes cell growth and eventually leads to the metastasis of lung cancer. The strategies employed for inhibiting EGFR include inactivation of the TK signaling cascade or the use of antibodies to neutralize the EGFR and its associated ligands. There have been several reports about drugs and monoclonal antibodies that have been successfully used against the EGFR. However, the major concern with these therapies is the eventual development of resistance by the receptor, which has necessitated combination therapies, using dual drug systems or drug-antibody systems [10, 37].

Table 8.2 Different receptors features expressed in lung cancer

No.	Receptor name	Family/class	Type of cancer	Role/remarks	Current drugs/target therapy	References
1	Epidermal growth factor receptor (**EGFR**)	ErbB family of receptors	SCLC, NSCLC	Triggers proliferation of malignant lung cells, promotes antiapoptosis, and lung cancer metastasis	Cetuximab, bevacizumab, gifitinib, erlotinib, icotinib, and afatinib	[10]
2	Folate receptor alpha (**FRα**)	Folate receptor (FOLR) family	NSCLC	FRα is an acquired marker for the proliferation of tumor cells, tumor biology, and for patient prognosis	Vintafolide or MK-8109, farletuzumab, FRA-specific CAR-modified T-cells	[11]
3	Human epidermal growth factor receptor 2 (**HER2/neu**)	ErbB family of receptors	NSCLC	Involved in signal transduction pathways, leading to cell growth, and differentiation	Afatinib, rapamycin, trastuzumab, and trastuzumab/paclitaxel combination	[12, 13]
4	TNF-related apoptosis-inducing ligand (TRAIL) death receptors DR4 & DR5	Tumor necrosis factor receptorsuperfamily (TNFRSF)	NSCLC	These are plasma membrane proteins containing the intracellular death domains essential for the transmission of the death signals upon binding of TRAIL	rhTRAIL	[14, 15]
5	Tetraspanins CD151	Transmembrane 4 superfamily (TM4SF) proteins	NSCLC	CD151 has a key role in cell proliferation, migration, colony formation, and signal transduction	CD151-siRNA, anti-CD151 mAb (1A5)	[16–18]
6	Mesenchymal–epithelial transition (MET)	Belongs to tyrosine kinase receptor family	NSCLC and mostly in lung adenocarcinoma	Amplification of MET leads to overexpression of HGFR, which is involved in cancer cell proliferation, migration, invasion, and metastasis. MET amplification commonly causes resistance of EGFR TKI	Therapies for MET are in clinical trial phase. Onartuzumab, a monoclonal antibody against MET has shown good results in phase II clinical trials. Crizotinib is a drug being developed against MET. Also, combination therapies with EGFR TKI, are being studied: erlotinib ± cabozantinib, erlotinib ± tivantinib, gefitinib + capmatinib, etc., are also used	[19, 20]

7	Estrogen receptors (**ERα and ERβ**)	Steriod hormone superfamily of nuclear receptors	NSCLC	ERβ activates PI3K/AKT/Bcl-XL and the RAS/RAF/MEK/ERK signaling pathways to regulate, cell proliferation, invasion, metastasis, mitochondrial biogenesis, and antiapoptosis	Fulvestrant, aromatase inhibitors, letrazole, exemestane, and tamoxifen	[21]
8	G-protein-coupled estrogen receptors	Steroid hormone superfamily of nuclear receptors	NSCLC	Involved in the cancer cell proliferation, migration, and invasion and acts as a modulator of the neoplastic transformation	Antagonists/modulators of the classical estrogen receptors, such as tamoxifen, raloxifen, and fulvestrant, were found to be the GPER agonists	[22]
9	Progesterone receptors (**PRA and PRB**)	Steroid hormone superfamily of nuclear receptors	NSCLC	Promotes tumor angiogenesis	Progesterone	[23]
10	Peroxisome proliferator-activated receptor gamma (**PPARγ**),	Nuclear receptor subfamily	NSCLC	PPARγ activationcan inhibit nuclear factor-κB and COX-2 expression in NSCLC	Rosiglitazoneand troglitazone, thiazolidinedione class (TZDs)	[24]
11	Somatostatin	G-protein-coupled receptor family	SCLC, NSCLC	SSTRs signal through phosphotyrosine phosphatases to induce apoptosis, as well as to decrease cell proliferation	Peptide-receptor radiation therapy (PRRT)	[25, 26]
12	Neuropilins receptor proteins (**NRP**)	Belongs to family semaphorins, which are associated with the secreted, transmembrane and GPI-linked proteins for axonal guidance and development of nervous system	NSCLC	NRPS are associated with increased tumor vascularization and poor diagnosis. These receptors interact with the VEGFs and other growth factors and also c-MET. Targeting NRPs results in both,greater vascular defects and death	Anti-NRP antibodies have been injected in animal models and have resulted in inhibition of tumor angiogenesis. Sulfated polysaccharideslike dextran sulfate and fucoidan have been used to induce internalization of NRP1. This blocks the function of SEMA3A and VEGF165 in the endothelial cells and promotes their internalization into the lysosomes	[3, 27–29]

(continued)

Table 8.2 (continued)

No.	Receptor name	Family/class	Type of cancer	Role/remarks	Current drugs/target therapy	References
13	Echinoderm microtubule-associated protein-like 4 -Anaplastic lymphoma kinase (**EML4-ALK**)	Belongs to tyrosine kinase receptor, a part of the superfamily of insulin	NSCLC	Overexpression in NSCLC due defect in the ALK receptor gene. It inhibits apoptosis, which promotes the proliferation of tumor cells	Crizotinib and ceritinib are FDA-approved drugs for the treatment in patients with ALK-positive lung cancer. Other drugs like brigatinib,alectinib, and lorlatinib are in clinical trial stages. However, limitation with these is that they are susceptible to resistance	[9, 30]
14	Vascular endothelial growth factor receptor (**VEGFR**)	Belongs to tyrosine kinase receptor	NSCLC	Overexpression in tumor promotes angiogenesis along with tumor progression	Monoclonal antibody bevacizumab is used to neutralize VEGFR isoforms. Further, dual therapy is used for inhibition of EGFR and VEGFR, using erlotinib and bevacizumab	[9, 31]|
15	Cluster differentiation 44 **(CD44)**	Transmembrane glycoprotein	NSCLC and squamous metaplasia	Progression of tumor with malignant and metastatic features. It also contributes to drug resistance in NSCLC	Various therapies are under review for CD44. But none of the therapies has made its way to the clinical trials. Interaction of CD44 with hyaluronan is under review along with RNAi technologies	[32, 33]
16	CD24	Cancer stem cell-associated membrane protein, a glycosylphosphatidyl-inositol-anchored molecule	Highly expressed in SCLC, but rarely in NSCLC	Increases cell invasion via biomechanical processes, increases tumor hypoxia by promoting production of H1F1α	Monoclonal antibody SWA11 and doxorubicin conjugated to SWA11	[34]
17	Bombesin receptors (**BBR1, BBR2, and BBR3**)	Group of G-protein-coupled receptors, which bind bombesin	SCLC, NSCLC	Involved in SCLC invasion and metastasis	GRPR antagonist RC-3095 with gemcitabine and temozolomide, cytotoxic agents like 5-FU, irinotecan, silencing of GRPR/GRP by siRNA-delivery	[34, 35]

18	Bradykinin receptors	Group of G-protein-coupled receptors	SCLC	Diverse functions including cell proliferation, leukocyte activation, cell migration, endothelial cell activation, and nociception	Bradykinin antagonist dimerCU201, in combination with doxorubicin, etoposide, cisplatin,vinorelbine, and paclitaxel increases SCLC growthinhibition	[34]
19	Oxytocin and vasopressin receptors	Tissue-specific G protein-coupled receptors	SCLC, NSCLC	ERK 1/2 phosphorylation and thesubsequent p90 ribosomal S6 kinase phosphorylation	Monoclonal antibody MAG-1	[34]
20	Insulin-like growth factor 1 receptor	Tyrosine kinase receptors	SCLC	Associated with apoptosis inhibition and proliferation stimulationthrough downstream signaling pathways including PI3K-Akt andMAPK	OSI-906	[34]
21	Fibroblast growth factor receptors	Members of the fibroblast growth factor family of proteins	SCLC	Promotes cancer progression, neoangiogenesis, and resistance to targeted treatments	Engineered soluble FGF receptor 1 Fc fusion protein, FP-1039 binds tightly to the mitogenic FGFs,inhibits FGF-stimulated cell proliferation, blocks FGF- and VEGF-induced angiogenesis and inhibits tumor growth FGFR pathway inhibition remains an active area of investigation in the SCLC and other tumors, in which this pathway is dysregulated	[34]

The EGFR is a 178 kDa transmembrane protein belonging to the receptor tyrosine kinase (RTK) family of proteins. The family consists of four members, namely, EGFR (Erb1, Her1), Erb2 (neu, Her2), Erb3 (Her3), and Erb4 (Her4). The receptor plays an important role in various cellular functions, including cell proliferation, survival, differentiation, and motility, and is necessary for the normal development of the organism [38].

3.1 Recognition Domain of the EGFR Receptor

All the aforementioned EGFR receptors share a basic structure, as depicted in Fig. 8.2, which consists of an extracellular binding domain that interacts with the ligands, the transmembrane domain traversing the lipid bilayer and the tyrosine kinase domain, on the cytoplasmic side, along with –COOH terminal tail containing several phosphorylation sites [39].

The extracellular region of the Erb family of receptors consists of 621 amino acids and includes two ligand-binding homologous domains (I and III) and two cysteine-rich domains (II and IV).

The transmembrane domain is made up of a single alpha helix containing 23 amino acids. The cytoplasmic domain consists of 542 amino acids that form the juxtamembrane cytoplasmic domain, a tyrosine kinase domain, followed by the carboxyl group terminal tail that encompasses multiple phosphorylation sites.

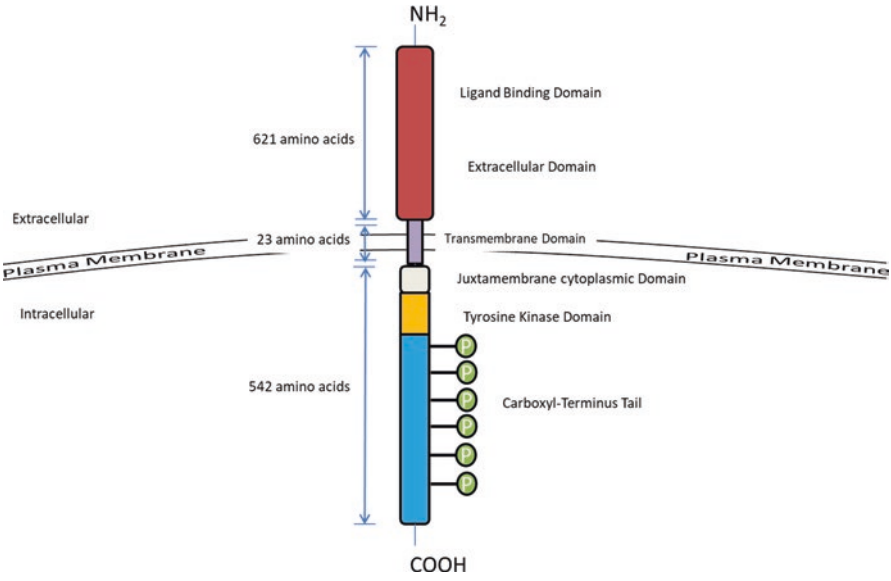


Fig. 8.2 The general structure of EGFR comprising the ligand-binding domain, transmembrane domain, tyrosine kinase domain, and carboxy terminal tail

The intracellular domain of the receptor has 20 tyrosine residues, out of which 12 are known to undergo phosphorylation. These phosphorylation sites serve as binding sites for the membrane-bound or soluble effector molecules, upon activation of the receptor.

Besides the membrane-bound forms, the Erb receptors are also found in soluble forms. The latter do not possess the transmembrane and the cytoplasmic domains and may be generated by proteolytic cleavage of the membrane-bound receptor or by alternative splicing [40].

Activation of the EGFR is controlled by its ligands. Upon binding with its ligand, a single molecule of EGFR dimerizes with another similar EGFR molecule (homodimerization) or with another member of the EGFR family (heterodimerization), preferably Erb2. Upon activation, the cytoplasmic side having the tyrosine kinase domains on both members of the dimer undergoes activation and is autophosphorylated at selective tyrosine residues in the tail region. The autophosphorylation sites serve as docking sites, directly or indirectly, for small signaling molecules such as Grb2, Grb7, Shc, Crk PLC-γ, SRC, PI-3K, and protein phosphatases – SHP1 and SHP2 and E3 ubiquitin ligase Cbl. Other molecules like STAT1, STAT3, STAT5, and PLD participate indirectly by playing a role in signaling. The activation of EGFR further stimulates several other pathways, which have been summarized in Fig. 8.3.

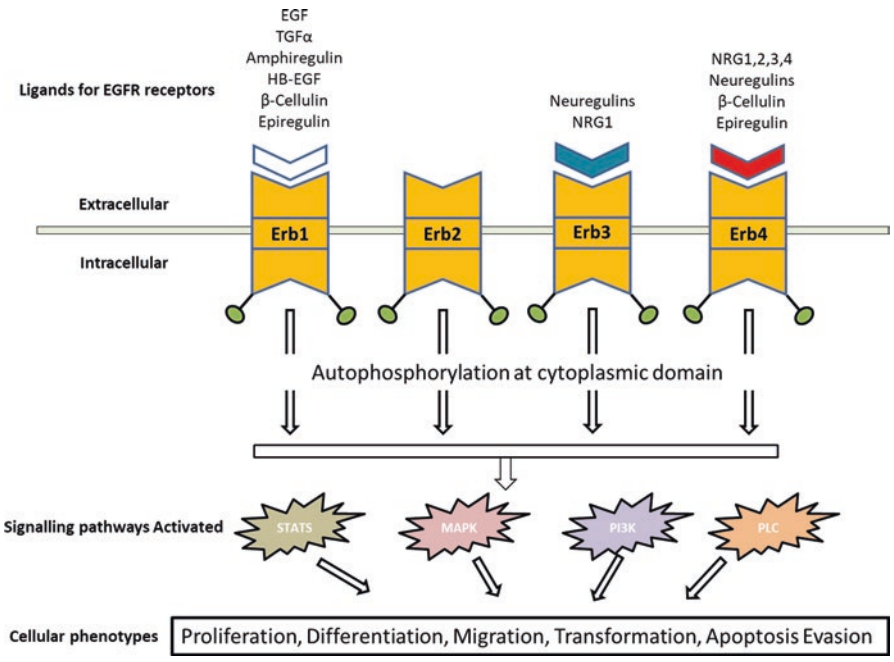


Fig. 8.3 The EGFR signaling network. MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3′-kinase; PLC, phospholipase C; STATs, signal transducers and activators of transcription

It is likely that the Erb2 receptor is necessary for the induction of tumor growth. The heterodimerization of Erb2 receptors with rest of the family members is an important mechanism for the oncogenic transformation of various types of tumors. This was studied using NIH3T3 cell line, which did not express any of the EGFR receptors. The influence of heterodimerization on tumor growth was assessed by transfecting various combinations of EGFR receptors in NIH3T3 cell line [41]. Those cells which expressed homodimers of Erb2, Erb3, and Erb4 did not induce any tumor growth, whereas cells which expressed only Erb1 had moderate tumorigenic characteristics. Interestingly, the Erb2/Erb3 pair was able to induce tumor growth, whereas the Erb1/Erb3 and Erb1/Erb4 pairs did not. On the contrary, the Erb1/Erb2 heterodimer pair was able to produce an aggressive tumorigenic phenotype in the NIH3T3 cells. Coexpression of Erb1 and Erb2 synergistically heightened the cellular response of the EGFRs and increased the overall expression of the proliferative markers [42].

In addition to this ligand-induced dimerization model for EGFR activation, EGFR can be activated by another model of ligand binding, known as the "rotational model." According to this model, the EGFR exists in an inactive, unliganded dimeric form. Once the ligand binds to the extracellular domain, a rotation is induced in its transmembrane domain, in a direction parallel to the plane of the lipid membrane of the inactive, dimeric form. This conformational change rearranges the intracellular kinase domain that leads to the conversion of the inactive symmetric receptor to an active asymmetric form.

3.2 Binding of Ligands with EGFR

As mentioned earlier, the dimerization of EGFR receptors is due to their ligands. There are 11 known ligands associated with the EGFR receptors. These can be divided into three groups, namely, (i) those that activate ErB1, namely, the epidermal growth factor (EGF), the transforming growth factor (TGF)-α, amphiregulin (AREG), and epigen (EPG), (ii) those which are formed by ligands that are bispecific to ErB1 and ErB4, namely, betacellulin (BTC), heparin-binding EGF (HB-EGF), and epiregulin (EPR), and (iii) those which are formed by neuregulins (NRG), which can bind to both, ErB3 and ErB4 (NRG1 and NRG3) or only ErB4 (NRG3 and NRG4). There are no known ligands that bind to the Erb2 receptor, which forms heterodimers with the other members of the EGFR receptor family, and its overexpression in cells causes ligand-independent cell transformation [43].

Each of these ligands has an EGF-like core domain, consisting of ~60 amino acids, which is responsible for facilitating their biological activity [44]. They are manufactured as type 1 transmembrane precursors that are usually cleaved from the extracellular domain to soluble forms, which then bind to the EGFRs and activate them. This cleavage is promoted by the proteins of a disintegrin and metalloproteinase family (ADAM), which form the soluble peptides, containing at least one EGF-like domain and spatially arranged cysteine residues, and are capable of EGFR

activation. These soluble factors bind to the EGFRs on the cells present at a distance from the release site (endocrine), the neighboring cells (paracrine) or the EGFRs present on the same cells (autocrine). Although the separation of EGFR-specific ligands seems to be an important step for receptor activation, many of the ligands such as HB-EGF, TGF-α, AREG, and BTC, are capable of activating the EGFRs even when they are hitched to the plasma membrane (juxtacrine) [40].

Upon ligand binding, the activated EGFR cluster is internalized via clathrin-coated, receptor-mediated endocytosis, where E3 ubiquitin ligase induces lysosomal degradation. The internal EGFR signaling and trafficking differs according to the various ligands of the receptor. HB-EGF and BTC signal continuous phosphorylation, ubiquitination, and degradation of EGFR. On the other hand, binding of TGF-α leads to temporary phosphorylation, minimum ubiquitination, and complete recycling of the endosomes containing the EGFRs. Inside the early endosomes, the TGF-α dissociates more readily from the receptor, due to the slightly acidic environment, which causes differential trafficking, and recycles the unbound EGFR back to the membrane [40, 45].

The EGFR receptors are internalized not only via clathrin-mediated endocytosis, but also via the caveolae. The mode of endocytosis is determined by the concentration of the ligand present, with a higher concentration inducing continuous phosphorylation and receptor degradation, leading to clathrin-independent endocytosis. On the other hand, lower ligand concentrations lead to clathrin-dependent endocytosis, along with receptor recycling [40].

3.3 Antagonists for Ligand Binding

Over the last decade, research in targeting of lung cancer, especially the NSCLC has been revolving around the use of two major receptor-targeting strategies. First is the use of immune inhibitors, namely, the anti-EGFR antibodies that bind to the extracellular domain and are highly specific for the receptor. The second strategy involves the use of small-molecule inhibitors that compete reversibly with the ATP to bind to the intracellular tyrosine kinase domain of EGFR, thus restricting autophosphorylation and blocking the downstream signaling. The mechanism of action and the biological effect of mAbs and small-molecule tyrosine kinase inhibitors (TKIs) depend on the route of administration, their bio-distribution, induction of EGFR downregulation, and activation of other immune functions. Despite their varied mechanisms of action, EGFR inhibition leads to some common antitumor effects such as inhibition of cancer cell proliferation by arresting the cell cycle in G0/G1 phase, induction of apoptosis, reduced production of the angiogenic growth factors, prohibition of cellular invasion and metastasis, and sensitization of the tumors to cytotoxic drugs and radiotherapy [46].

Among the anti-EGFR mAbs such as cetuximab, specifically bind to the extracellular region of the EGFR in its inactive form and thus obstruct the ligand-binding sites and block the activation of tyrosine kinase [47]. Apart from blocking the

signaling pathways, mAbs also display antitumor action through antibody-mediated cellular cytoxicity (ADCC) and complement-mediated toxicity [48].

Cetuximab is one of the most extensively studied anti-EGFR antibodies for targeting advanced NSCLC. It is a chimeric human murine IgG1 monoclonal antibody, obtained from the myeloma cell line. It consists of murine Fv EGFR-binding region and human IgG1 heavy and light chain Fc regions, collectively having an approximate molecular weight of 152 kDa. It binds to the ligand-binding domain III of the EGFR, with a high affinity (dissociation constant Kd of 1.8 nM, ~10-fold higher than its ligand), and thereby restricts the activation of downstream intracellular signaling, particularly mitogen-activated kinases pathway, by inhibiting the receptor dimerization. It has been observed in certain studies that cetuximab enhances the cellular internalization of the receptor thereby reducing the number of receptors available for ligand binding [49].

Other early competitors of cetuximab included panitumumab and matuzumab. However, they failed in phase II clinical trials as their combination with chemotherapeutic agents did not demonstrate a benefit to patients compared to the chemotherapy alone [50]. Other antibodies targeted toward EGFR include nimotuzumab, pertuzumab, trastuzumab, and necitumumab. Necitumumab has been recently approved by the USFDA for the treatment of squamous cell lung cancer, based on the results of the SQUIRE trial [51]. Its role in targeting of lung cancer, as well as the clinical efficacy in targeting various lung cancer conditions, has been elaborated [52].

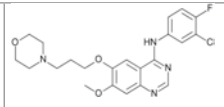
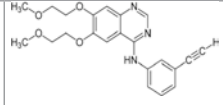
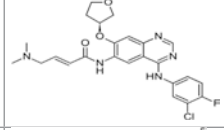
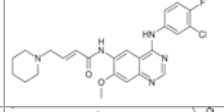
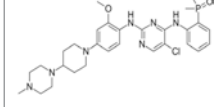
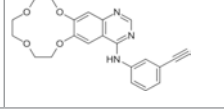
Among the TKIs, gefitinib (ZD1839, Iressa), was the first drug developed to inhibit the EGFR. This molecule competes reversibly with ATP to bind to the intracellular domain of the EGFR and blocks autophosphorylation and downstream signaling. It is an orally administered, low molecular weight, anilinoquinazoline tyrosine kinase inhibitor. A dose of about 250 mg/day is administered for the inhibition of the EGFR and its downstream signaling processes [53]. Gefitinib selectively binds to the EGFR tyrosine kinases and does not inhibit serine threonine kinases [54]. It is capable of arresting the cell cycle in the G1 phase and it reduces the levels of important angiogenesis factors like the VEGF [55, 56].

Other EGFR TKIs include erlotinib, icotinib, afatinib, dacomitinib, osimertinib, rociletinib, brigatinib, olmutinib. Out of these, GILOTRIF (afatinib), IRESSA (gefitinib), TAGRISSO (osimertinib), TARCEVA (erlotinib), VIZIMPRO (dacomitinib) have been approved for the first-line treatment of NSCLC with EGFR mutation.

3.4 Significant Inhibitors of EGFR

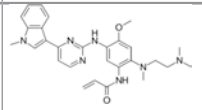
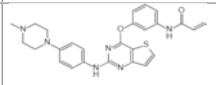
Till date, various drugs/inhibitors have been evaluated for their therapeutic effect in lung cancer. These have been enlisted in Table 8.3, along with their mechanisms of action and structures.

Table 8.3 Inhibitors/drugs for the treatment of lung cancer

Sr.No	Compound	Company	Structure	Mechanism of action	Developmental phase	References
Small molecule: tyrosine kinase inhibitors (TKI)						
1	Gefitinib	AstraZeneca/Teva		First inhibitor of EGFR tyrosine kinase; inhibits by binding to the adenosine triphosphate (ATP)-binding site of the enzyme; this leads to the inhibition of the Ras signal transduction pathway which is "antiapoptotic"; thus, the malignant cells are arrested	Approved	[57]
2	Erlotinib	Genentech/OSI Pharmaceuticals/Roche		Specific inhibitor of (EGFR) tyrosine kinase binds to the ATP-binding site in a reversible manner that inhibits the formation of phosphorylated residues in EGFR and arrests the signaling cascade	Approved	[58, 59]
3	Afatinib	Boehringer Ingelheim GmbH		First-generation TKI, irreversibly inhibits the EGFR by covalently binding to the cysteine 797 of the EGFR It also acts on the T790M mutants of the NSCLC	Approved	[60]
4	Dacomitinib	Pfizer		It is selective to EGFR and binds irreversibly to the receptor	III	[61]
5	Brigatinib	ARIAD Pharmaceuticals		First-generation potent ALK inhibitor of the target protein and of the mutant. Second-generation EGFR inhibitor that acts by deactivating the EGFR or its T790M mutant	III	[62]
6	Icotinib	Beta Pharma		First-generation TKI for the EGFR. Inhibits by competing against the ATP; binds to the ATP-binding site reversible and deactivates the EGFR	Approved	[63]

(continued)

Table 8.3 (continued)

Sr.No	Compound	Company	Structure	Mechanism of action	Developmental phase	References
7	Osimertinib	AstraZeneca		Third-generation EGFR TKI. It binds irreversibly to EGFR proteins expressed by the EGFRs with a T790M mutation; also binds irreversibly to EGFRs with L858R mutation and with an exon 19 deletion	Approved	[64]
8	Olumitinib	Hanmi Pharmaceutical/ BoehringerIngelheim		It is the second-line treatment for NSCLC, having T790M mutation of EGFR It covalently binds to the cysteine residue near the kinase domain of the EGFR	Approved	[65]
Monoclonal Antibodies (MAb)						
1	Cetuximab	Bristol-Myers Squibb/ Merck KGaA	Chimeric (mouse/ human) monoclonal antibody	Inhibits EGFR by interfering with the KRAS signaling cascade	Approved	[66]
2	Zalutumumab	Genmab	Fully human IgG1 monoclonal antibody	It binds to the EGFR Domain III on the cell surface. This locks the receptor in an inactive conformation, competing against the EGF, thus arresting dimerization and promoting apoptosis. It has also has shown ADCC activity	III	[67]
3	Nimotuzumab	Biocon/TheraCIM/ CIMYM Biosciences/ Theraloc/CIMAher	Humanized monoclonal antibody	Its binding affinity is toward the extracellular region of the EGFR, which blocks the ligand-binding region and thus arrests the EGFR activity.	III	[68]
Vaccines						
1	CimaVax-EGF	Centre of Molecular Immunology, Havana	Recombinant human EGF conjugated to a protein carrier	It is an active vaccine that produces antibodies against the EGF	I/II	[69]

3.5 Receptor-Mediated Targeting Strategies

Although various types of lung cancers have been treated using different mAbs and small-molecule TKIs, new strategies for actively targeting the EGFR are being researched by scientists around the globe.

3.6 Prodrugs/Drug Complexes

Prodrugs are medicines or compounds that upon administration are metabolically converted into pharmacologically active drugs. A prodrug can be designed to selectively interact with the cells or processes that are not its direct targets. It may help to improve the specific availability of the drug at the disease site and thus reduce the associated adverse effects.

Prodrugs have been widely used in targeted drug delivery systems to unload the cytotoxic compound into the tumor cells. They offer various strategies for their activation chemistry and can thus act against diverse types of cancers. The current trends in the development of prodrugs for cancer therapy include the use of macromolecules, such as drug-antibody conjugates, polymer-drug conjugates, and other self-assembling macromolecules, such as lipids that form liposomal or micellar nanoparticles. Various chemotherapeutic agents, including paclitaxel, doxorubicin, carboplatin, etc. have been conjugated with polymers, such as PLGA, PEG, etc. to synthesize prodrugs for different types of lung cancer.

The two strategies used for the conversion of prodrugs into active drugs include (i) passive approaches that exploit the basic physicochemical or physiological changes (for e.g., reduced pH, hypoxia, overexpression of the surface receptor) and (ii) active strategies that utilize prodrugs that may be activated by a site-directed enzyme, thus aiding in specialized activation chemistry for the prodrug conversion.

Many enzymes are known to be upregulated in cancer. DT-diaphorase (DTD) is elevated in many cancers including NSCLC [70]. This is a cytosolic enzyme that reduces two electron containing quinone substrates and activates mytomycin C, the DNA cross-linker. DTD can be targeted by alkylating agents, such as RH1 that causes the bioreduction of the attached quinone to selectively activate the aziridine rings in the cancer cells [71]. Also, cytosolic phospholipase A2α, which plays an important role in cell cycle regulation, has been targeted by researchers. Elevated levels of PLA2α increase the production of eicosanoids that results in the promotion of tumor growth and metastatic activity of the tumor. Further, its inhibition is known to suppress the proliferation of tumor cells by inducing apoptosis. Subsequently, a nanodrug delivery system consisting of mesoporous silica nanoparticles containing pyrrolidone-2, and decorated by EGFR receptor-targeted antibody (EGFRAb) was developed. Silica nanoparticles (SN) are nontoxic and pyrrolidone-2, a potent inhibitor of PLA2α, blocks the production of prostaglandins E2 and leukotriene. EGFRAb

was employed to direct the silica nanoparticles specifically to the cancer cells. In vitro studies in H460 lung cancer cells showed the potency of pyrrolidone-2-loaded SN-EGFRAb nanoparticles, by reducing the activity of PLA2α, decreasing the levels of arachidonic acid and limiting the cell proliferation. Furthermore, this nanoparticulate system showed better antitumor activity (38%) with enhanced tumor inhibition rate in a subcutaneous model of NSCLC. Also, the EGFR antibody helped in targeting the nanoparticles specifically to the tumors cells as compared to the native nanoparticles [72].

The second approach involves the passive targeting of prodrugs to the tumor. This approach exploits the physicochemical characteristics of the cancer cells, such as the tumor microenvironment. The solid tumors in general are hypoxic due to deregulated cell growth and poor vascularization. Due to the hypoxic conditions, the cancer cells resist cell death, induce angiogenesis and interfere with energy metabolism of the cells. This enhances the cancer aggressiveness and metastasis. Under such conditions, due to falling oxygen levels, an important transcription factor called hypoxia-inducible factor-1α (HIF-1α) promotes the expression of genes responsible for the suppression of apoptosis, angiogenesis, invasion, and motility [73]. In NSCLC, HIF-1α expression causes resistance to radiotherapy, chemotherapy, and EGFR TKIs [74–77]. Since hypoxia is connected to the resistance in NSCLC therapy, researchers have targeted cancers using hypoxia-activated prodrugs (HAP). One classic example of HAPs is that of tarloxotinib bromide, a bioreductive pan-EGFR inhibitor. Under hypoxic conditions, tarloxotinib undergoes metabolism via one-electron reduction to a fragment and releases a potent EGFR TKI that exerts antiproliferative activity. Tarloxotinib bromide was designed to release an EGFR TKI, erlotinib under hypoxic conditions. Efficient metabolism of tarloxotinib was demonstrated in a range of human NSCLC cell lines and it was shown to be more effective than erlotinib in wild-type and EGFR-mutant NSCLC xenografts [78, 79]. Targeting cancer with tarloxotinib/erlotinib had reached phase II clinical trials in patients with NSCLC; however, poor response rates led to the discontinuation of these trials [80].

Recently, scientists have designed and synthesized an active tumor targeting prodrug, gefitinib (PPG), which is a polyamine analog, for precision therapy in NSCLC. This macromolecule containing an EGFR TKI was not only successful in inhibiting the growth of PPG-sensitive PC9 cells, but was also efficient in killing the PPG-resistant H1650 cells [81].

3.7 Nanocarriers Targeting EGFR Receptor for Lung Cancer Therapy

The nanocarrier approach offers the ability to target the drugs accurately to the tumorous tissue, which may reduce the toxicity of chemotherapeutic agents. Nanoparticles can be targeted via active or passive approaches. Three types of nanoparticles have been explored for the treatment of lung cancer, namely, (1)

natural nanoparticles (2) organic nanoparticles, and (3) inorganic nanoparticles. Of these, many have been used to target the EGFR for specific delivery of various therapeutic compounds and have been listed in Table 8.4.

4 The Receptor: c-MET

c-MET receptor is overexpressed in lung cancer as an outcome of the resistance developed through the EGFR inhibitors, which leads to c-MET amplification. Therefore, c-MET is an important receptor in NSCLC. It is a transmembrane tyrosine kinase receptor (RTK), which is activated by the ligand, the hepatocyte growth factor (HGF). Activation of c-MET RTK drives a plethora of molecular events in the cells, thus rendering it as an ideal target for therapy. Amplification of c-MET in NSCLC leads to proliferation, invasion, metastasis, and angiogenesis of the cancerous cells. As NSCLC has a poor prognosis and is highly malignant due to the overexpression, amplification, and association of c-MET, the receptor can act as a useful target for treating this cancer type. Thus, various therapies and drugs targeting c-MET are currently being tested either alone or in combination with monoclonal antibodies. Various monoclonal antibodies like emibetuzumab, ficlatuzumab, and rilotuzumab along with tyrosine kinase inhibitors, (TKI) such as crizotinib, tepotinib, cabozantinib, and capmatinib, are currently under evaluation. These studies have resulted in an improvement in the overall survival rate of NSCLC patients [80, 89]. Further, investigators have explored c-MET and EGFR for developing combination therapy against NSCLC, as c-MET is known to have considerable cross-talks with the other signaling pathways. Therefore, a comprehensive study of this receptor is anticipated to impart significant knowledge regarding its role in NSCLC [20].

4.1 Recognition Domain of c-MET

c-MET or the hepatocyte growth factor receptor (HGFR) is a protein tyrosine kinase like the EGFR and belongs to the family of oncogenes that regulate important cellular processes, such as differentiation, proliferation, cell cycle, motility, and apoptosis [90].

c-MET is a transmembrane receptor tyrosine kinase (RTK), which is a 150-kDa polypeptide. Upon glycosylation, the receptor is activated and a forms 190 kDa glycoprotein. The receptor comprises a transmembrane β-chain (140 kDa) that is extracellularly attached to the α-chain (50 kDa) via a disulfide linkage. This constitutes the binding site for the ligand at the N-terminal of the c-MET receptor [20, 91, 92]. The receptor is activated by its ligand, namely, the hepatocyte growth factor (HGF), which is a member of the plasminogen-related growth factor family. The precursor of HGF is mainly produced by the cells of mesenchymal origin. There are

Table 8.4 Summary of nanocarriers conjugated with various anti-EGFR molecules for the treatment of lung cancer

Sr. No.	Nanoparticle material	Targeting molecule	Cell line or animal model	Remarks	Ref
1	Gold nanoparticles as contrast agents	Cetuximab (C225) and Llama heavy chain variable region antibody fragments (VHH domains)	4–6-week-old female athymic nude mice were injected with A431 cells to develop tumors having volume of 5 cc	The nanoparticle system allowed effective tumor imaging by computed tomography (CT) with enhanced uptake because of cetuximab	[82]
2	Silica nanoparticles	Anti-EGFR monoclonal antibody	In vitro studies (A549) and BALB/c nude mice induced with A549 tumors	Silica nanoparticles (~100 nm) containing mAb and methylene blue complex were developed as probes for lung cancer detection	[83]
3	Gold nanoparticles	C225	Xenograft models prepared with nude mice injected with A549 and H1299 cells	Gold nanoparticles (~14 nm) efficiently delivered C225 and increased the cytotoxic effect in EGFR-positive NSCLC	[84]
4	Chitosan nanoparticles cross-linked with γ -poly(glutamic acid) loaded with Docetaxel	C225	A549 cells	The drug delivery system showed superior antiproliferative activity over untagged docetaxel chitosan nanoparticles. The cell cycle was arrested in G2/M phase and resulted in the induction of apoptosis	[85]
5	PLGA Nanoparticles loaded with Docetaxel	C225	A549 cells and xenograft mice models bearing A549 tumors	Sustained cytoplasmic delivery of docetaxel was achieved	[86]
6	Liposomes loaded with doxorubicin	GE11 (short peptide specific to EGFR)	A549 cells, male BALB/c nude mice induced with A549 tumors	Liposomes with 10% GE11 had the highest tumor cell killing activity and a 2.6-fold lower IC50 than that of the nontargeted carriers. GE11-modified liposomes showed enhanced accumulation and prolonged retention in tumor tissue	[87]
7	1,2-distearoyl-sn -glycero-3-phosphoethanolamine-N-(amino(polyethylene glycol)-2000 (PEG 2000/ DSPE)	Human epidermal growth factor (EGF)	A549-T24 human lung adenocarcinoma cells	Cellular uptake study revealed that EGF-targeted micelles afforded higher intracellular delivery of paclitaxel as compared to the nontargeted micelles in both resistant and sensitive cell lines	[88]

six domains in the HGF, namely, the N-terminal domain, four kringle domains and the C-terminal domain, which is a catalytic domain that is structurally similar to the serine proteases. The HGF binds to the c-MET in 2:2 ratio, that is, two HGFs bind to the dimerized form of c-MET/HGFR [93], via the semaphorin domain at the N-terminal. The tyrosine kinase domain is located intracellularly in the β chain near the C-terminal end. This end is essential for binding to the substrate and subsequent downstream signaling [92]. The binding of the HGF to c-MET is known to activate several signaling cascades like the growth factor receptor-bound protein 2 (Grb2), mitogen-activated protein (MAP) kinase, phosphoinositol 3-kinase (PI3K), and phospholipase C-γ (PLC-γ). This receptor–ligand interaction is known to control morphogenesis, motility, mitogenesis, and proliferation in epithelial and endothelial cells [94, 95]. These pathways can promote cell survival and proliferation along with migration, motility, invasion, and angiogenesis, and can bring about transition of epithelial cells to mesenchymal cells [20, 96].

4.2 Interaction of the Receptor with Ligands

The receptor contains an extracellular region, the semaphorin domain that is a cysteine-rich immunoglobulin domain and an intracellular juxtamembrane domain, a tyrosine kinase catalytic domain and a carboxy terminal docking site (Fig. 8.4) [20, 96]. The HGF binds to the c-MET at the N-terminal domain, which is also known as the semaphorin domain or the sema domain. The sema domain is made up of seven β sheets that form a bladed propeller structure having seven arms. Here, the second and the third sheets bind to the active site region of the ß-chain of HGF

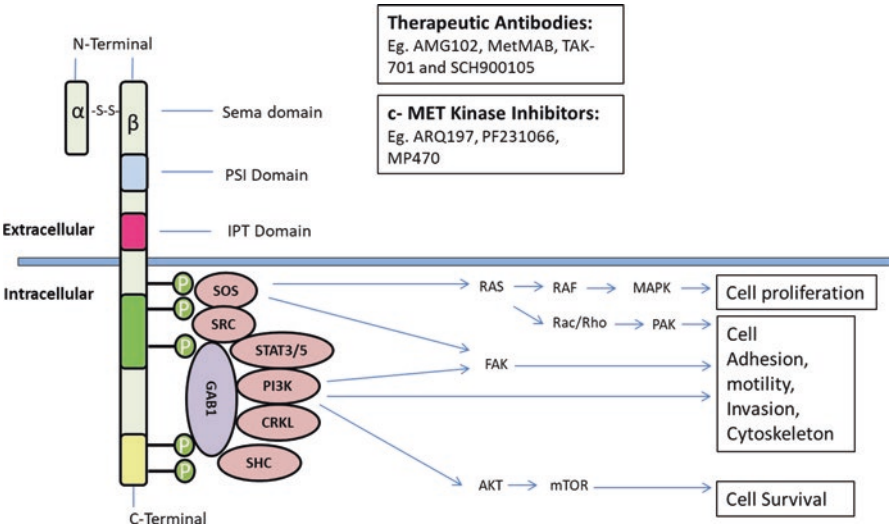


Fig. 8.4 Activation of c-MET and signaling cascade associated with its activation

[97, 98] The tyrosine kinase (TK) region is present at the C-terminal domain, which envelopes multiple sites for the phosphorylation of tyrosine. Upon binding with the c-MET receptor tyrosine kinase, the HGF activates the receptor by dimerization. Homodimer formation further transactivates the TK and the juxtamembrane domains. This cross-transactivation results in autophosphorylation of the three conserved tyrosine residues in the activation loop of the TK domain of the c-MET receptor [99, 100].

Phosphorylation of the c-MET receptor can be mediated either via the HGF or via various RTKs. This further activates various signaling cascades, which bring about changes at the molecular level, through the recruitment of various proteins that play a role in signaling. These signaling cascades govern various biological actions, such as regulation of transcription and gene expression, survival, reduced apoptosis, and regulation of cytoskeletal function along with cell growth and differentiation [90, 101].

However, the main difference in the expression of c-MET in normal and oncogenic cells is that the receptor activation is mediated through the ligands only in case of normal cells, which does not occur in the oncogenic cells [93].

4.3 Antagonists for c-MET

The molecules that mimic the HGF are natural antagonist of c-MET. These include modifications of the HGF that have shown antagonistic activity against the natural HGF. These molecules compete for the binding site at the c-MET without bringing about the required conformational change required for dimerization during the receptor activation. The most common molecule is the pro-HGF, which is also the precursor of HGF and is known to bind to the receptor without bringing about its activation. Further, NK2 and NK4, the HGF α-chain variants, bind to the c-MET without activating the receptor and thus act as antagonists. The NK2 and NK4 consist of a hairpin N-terminal domain and 2–4 kringle domains (2 in case NK2 and 4 in case of NK4) which compete with the HGF. NK2 may act as an antagonist or partial agonist to c-MET, and occurs naturally. On the other hand, NK4 is produced by the proteolytic digestion of HGF and has exhibited a better therapeutic value since its structure is similar to angiostatin that downregulates angiogenesis [89, 102, 103]. Along with these antagonists, there are certain c-MET decoys that have an ability to inhibit the receptor. The above-discussed are naturally available ligands for c-MET.

4.4 Ligands of c-MET

Most of the synthetic ligands of the c-MET receptor target the DFG motif. The DFG motif comprises aspartic acid (D), phenylalanine (F), and glycine (G) residues. It is present at the N-terminal, near the “activation loop” that covers the catalytic site,

the latter being important for the regulation of the receptor. A conformational change in the receptor modulates the kinase activity from DFG-in to DFG-out, that is, active to inactive state [104]. This property of the receptor has been explored for its inhibitory action and has resulted in three main classes of c-MET inhibitors that differ in their structure–activity relationship. The class of small molecules that act on DFG-in state are termed as Class I inhibitors, comprising small molecules like PF-2341066 (Pfizer) and SU11274 (Sugen) [105, 106]. The Class II or AM-like inhibitors bind to the inactive state of DFG-out. These are mainly derived from urea and are either ring-based or non-ring-based structures [107]. They interact with the hydrophobic pocket in the region between the hinge and the C-helix, thus assuming an unphosphorylated conformation of c-mET. These two classes of compounds are competitive ATP inhibitors. Majority of the inhibitors target via competitive inhibition; however, noncompetitive ATP inhibitors have also been explored. ARQ197 (Tivantinib) is a small molecule that inhibits c-MET by interfering with the ATP binding noncompetitively. In vitro, this small molecule binds to dephosphorylated c-MET and is a bisindolylmaleimide. However, its exact mechanism of action still remains unclear, though it has been observed to be safe to the cells [108]. Various ligands have been explored for inhibiting the c-MET receptor and have been listed in Table 8.5. The inhibitors of c-MET mostly compete for ATP-binding sites either in a competitive or noncompetitive manner.

Table 8.5 Ligands explored for inhibition of c-MET

No.	Compound	Developer	Mode of action	Development phase	References
1	ARQ197	ArQule/ Daiichi Sankyo	Noncompetitive; selective, mechanism not clear. Administered with erlotinib for NSCLC	II	[109]
2	PF-2341066	Pfizer	ATP-competitive; c-MET and ALK inhibitor	III	[105]
3	PF-4217903	Pfizer	ATP-competitive; selective	I	[110]
4	JNJ-38877605	Johnson & Johnson	ATP-competitive; selective; for solid tumors	I	[111]
5	XL184/ BMS907351	Exelixis/ BMS	Nonselective inhibitor of tyrosine kinase, effective against c-MET in cases of NSCLC	II	[112]
6	AMG102/ rilotumumab	Amgen	Humanized antihuman HGF IgG2 for SCLC and adenocarcinoma	II	[113]
7	MetMAb	Roche	Humanized antihuman c-MET monovalent antibody, for NSCLC	II	[114]
8	AMG-458	Amgen	ATP-competitive, c-MET, and Ron inhibitor	Preclinical	[115]

4.5 Receptor-Mediated Targeting Strategies

A drug's efficiency is determined by its ability to target the specific site of action. Currently, various nanocarrier-based systems are being explored to enhance the receptor-targeting efficiency. However, only a few systems targeting c-MET have been designed for the therapy of lung cancers as their therapeutic status in progression of lung cancer is still being investigated.

A novel theranostic system was developed by Lu et al. wherein the researchers conjugated quantum dots with human single chain variable fragment (scFv) antibodies. The scFv antibody targeted against c-MET was used to decorate the surface of PEGylated liposomes for delivering doxorubicin in in vitro and in vivo investigations. These liposomes could selectively deliver the drug for treating metastases of lung cancer [116]. Further, another system comprising an adenoviral vector, along with the RGD cell-penetrating peptide, induced with NK4 antagonist of HGF, in mesenchymal stem cells. When this system was tested in a murine model of lung metastasis (C-26), it resulted in an increase in the survival rate of the treated mice. Thus, this drug delivery carrier was able to reduce angiogenesis in tumors and induced apoptosis in the tumorigenic cells, thus prolonging the survival of C-26 mice. The system was thus proposed for the treatment of multiple lung metastatic cancer [117]. Similar therapies have been used for treating solid tumors, glioblastomas, and hepatocytic carcinomas due to the upregulation of c-MET observed in these cancers. As c-MET is a pleiotropic receptor, therefore, inhibitors of c-MET give best results when used in combination with other receptor inhibitor drug. Thus, combination therapy can help to overcome the drug resistance along with arresting of metastasis.

5 VEGF (Vascular Endothelial Growth Factor)

VEGF is a heparin-binding homodimeric glycoprotein, which belongs to the family of growth factors. VEGF exerts its action through the interaction with two highly related tyrosine kinase receptors, VEGFR1 and VEGFR2, which are predominantly expressed in cancer cells. VEGF is the main driver of angiogenesis and is overexpressed NSCLC [31]. A variety of environmental factors (hypoxia), growth factors, and genetic/epigenetic factors (oncogenes/tumor suppressor genes) regulate the expression of VEGF in lung cancer. Along with cytokines and metalloproteinases, the transforming growth factor beta 1 (TGFβ1) and tumor necrosis factor alpha (TNFα) also stimulate the production of VEGF in the lung cancer cells. The NSCLC cells can produce and secrete VEGF, promoting the formation of pleural effusion, angiogenesis, and tumor metastatic progression. Current strategies of inhibiting the VEGF pathway include two main approaches, monoclonal antibodies for targeting the VEGF or VEGFRs and tyrosine kinase inhibitors. Currently, bevacizumab and ramucirumab have been approved for treating the NSCLC patients receiving chemotherapy. On the other hand, the tyrosine kinase inhibitor, nintedanib, in combination

with docetaxel, is the only multikinase antiangiogenic agent that has been approved for treating lung cancer patients with advanced adenocarcinoma, after first-line chemotherapy. Thus, targeting VEGF is foreseen as a promising strategy for the treatment and diagnosis of lung cancer [31, 118].

VEGF plays an important role in tumor development by mediating angiogenesis. It is highly expressed in tumor cells and has implications in both NSCLC and SCLC [119]. The main function of VEGF is to promote tumor growth through neoangiogenesis, lymphangiogenesis, and lymph nodal dissemination. The structure, function, ligand binding, and recognition domain of this receptor have been elaborately discussed separately (Chap. 8). Here, we have discussed about the role of VEGF in lung cancer and how this receptor may be employed as a therapeutic target for treating lung cancer.

5.1 Natural and Synthetic Ligands for VEGFR

Various ligands have been explored for inhibiting the VEGFR. Their mode of action, current developmental phase, and the companies involved in their development have been stated in Table 8.6.

Table 8.6 Various ligands explored for inhibition of VEGF in their developmental phase for lung cancer [120, 121]

No.	Compound	Developer	Mode of action	Developmental phase	References
1	Bevacizumab	Avastin; Genentech	Recombinant humanized IgG1 mAb. It blocks angiogenesis by inhibiting VEGF-A	Approved	[122]
2	Ramucirumab	ImClone Systems Inc.	Fully human IgG1 monoclonal antibody targeting the extracellular domain of VEGFR-2	Approved	[123]
3	Sorafenib	Bayer	Inhibits RTKs including VEGFR	III	[124, 125]
4	PTK787 (Vatalanib)	Novartis	Oral inhibitor of VEGFR-1, -2, and -3 tyrosine kinases	III	[126]
5	Cediranib	AstraZeneca	Inhibits VEGFR-1 and/or VEGFR-2; multikinase inhibitor that has been studied as the first-line therapy for advanced NSCLC	II/III	[127, 128]
6	Nintedanib	Boehringer Ingelheim	Potent TKI having anti-VEGFR-2 activity	III	[129]
7	Neovastat (AE-941)	Æterna	Inhibits VEGF binding and VEGF TK activity	III	[130]

5.2 *Receptor-Mediated Targeting Strategies*

siRNA has proven to be a promising molecule for treating various cancers. However, the molecule requires a robust delivery carrier owing to its extreme fragility. Antiangiogenic therapies were designed by Kim et al., where antiangiogenic siRNA was conjugated with nanoparticles having polyethylenimine (PEI) core and a PEG shell. This was employed for downregulating VEGF expression in animal tumor models. This system was effective in treating lung cancer systemically and locally [131]. Another antiangiogenic system was designed to contain docetaxel and an anti-VEFG intraceptor, and was further decorated with RGD peptide for cell penetration. This combination therapy was tested in H1299 lung cancer cells and in xenografts in athymic nude BALB/c mice. This combination therapy resulted in a higher inhibition of VEGF, promoted apoptosis and arrested angiogenesis [132]. Further, nanocarriers were effectively used for delivering a highly hydrophobic drug, possessing known multitarget antiangiogenic effects. Here, albumin nanoparticles were developed along with polymeric micelles and were administered together. The polymeric micelles resulted in a strong inhibition of angiogenesis, while the albumin nanoparticles demonstrated retardation of tumor growth. Thus, the dual carriers provided a novel combination therapy for tumor regression [133].

6 Drug Resistance in Lung Cancer

Lung cancer is often associated with unprecedented reoccurrence of the disease, probably due to the ineffectiveness of the therapies, most of which are associated with drug resistance. Most of the EGFR-mutant NSCLCs actively respond to the EGFR inhibitors. But, a vast majority of these tumors ultimately become resistant to the drug treatment. About 50% of this resistance is due to the occurrence of a secondary mutation in EGFR (T790M) [134–137]. The T790M mutation mostly occurs due to the first-generation EGFR inhibitors. This mutation is also referred to as the “gatekeeper” mutation [136]. Further, this mutation also triggers MET amplification, which signals through ERBB3 and is characterized by gene amplification of a kinase that is not a direct or downstream target of gefitinib or erlotinib [136]. These findings may have important clinical implications for patients who develop acquired resistance to gefitinib, erlotinib, and afatinib. Hence, combination therapies involving MET kinase inhibitors and irreversible EGFR inhibitors have been recommended for patients whose tumors become resistant to gefitinib or erlotinib [134]. Regales et al. have suggested that dual targeting with cetuximab and a second-generation EGFR TKI can effectively overcome the T790M-mediated drug resistance. Though the combination of afatinib and cetuximab is associated with a response rate of 29% (32% among patients with EGFR T790M and 25% among patients without it), it is associated with side effects such as substantial skin toxicity (20% of grade 3 or higher) and gastrointestinal toxicity (6% of grade 3 or higher)

[135]. AZD9291 is an oral, potent, irreversible EGFR tyrosine kinase inhibitor, developed by AstraZeneca that is selective for the EGFR tyrosine kinase inhibitor–sensitizing mutations and the T790M resistance mutation. The USFDA approval was granted to this drug after it demonstrated efficacy in 411 NSCLC patients with T790M mutations, who exhibited an overall objective response rate (ORR) of 59%. AZD9291 is a monoanilino-pyrimidine compound that is structurally distinct from the other third-generation EGFR TKIs and offers a pharmacologically differentiated profile from the previous generation EGFR TKIs. During the preclinical studies, this drug has been shown to potently inhibit the signaling pathways and cellular growth in both EGFRm+ and EGFRm+/T790M mutant cell lines, in vitro studies. A lower activity against was reported with this molecule in wild-type EGFR cell lines, translating into profound and sustained tumor regression in EGFR mutant tumor xenograft and transgenic models [137].

7 Combination Therapy in Lung Cancer

Lung cancer can be activated through the upregulation of multiple receptors that are responsible for regulating numerous pathways and hence treatments employing monotherapies have been observed as largely ineffective. Thus, combinatorial therapies that simultaneously target different pathways have been foreseen to be promising for treating various forms of this cancer [138]. Combination therapies rely on combining two or more anticancer drugs with the purpose of eliminating the cancer cells. Such an approach is advantageous because the drug combination acts in a synergistic or additive manner on the key target pathways responsible for cancer phenotypes.

The platinum-based chemotherapy is the first-line approach for patients with advanced NSCLC, which results in a median overall survival rate of 8–12 months. Biological molecules, such as bevacizumab and cetuximab, have led to only modest differences in the survival, which has necessitated newer therapeutic paradigms [139]. Paclitaxel/carboplatin has been regarded as a standard drug for combination therapies due to their frequent usage and efficacy in NSCLC patients. Lynch et al. assessed the activity of ipilimumab, which is an anticytotoxic T-cell lymphocyte-4 monoclonal antibody in patients with lung cancer. A randomized phase II study was conducted to compare ipilimumab along with paclitaxel and carboplatin as compared to the drugs combination alone [140]. The study resulted in an improved immune-related progression-free survival rate in patients receiving ipilimumab as compared to those receiving the drug combination without ipilimumab (median 12.9 vs. 9.9 months) [139, 140]. Pirker et al. conducted a phase III study to assess the efficacy and safety of the EGFR-targeted monoclonal antibody, cetuximab in combination with cisplatin/vinorelbine (CV) and compared the effects in NSCLC patients receiving only CV. They found that the combination of cetuximab with CV resulted in superior survival of the patients with advanced EGFR-detectable NSCLC [141].

Another study involved combination therapy with trastuzumab and pertuzumab in Calu-3 and KPL-4 xenograft models. This resulted in tumor regression and a complete inhibition of metastatic tumor spread in animals. Pertuzumab is a HER2 dimerization inhibitor that binds to a different epitope on HER2 than trastuzumab and inhibits the formation of dimers of HER2 with other HER family members, such as HER3 and HER1. The combination of trastuzumab and pertuzumab demonstrated enhanced antitumor effects and promoted tumor regression in xenograft models of HER2-positive breast cancer and NSCLC. Although both these agents could actively induce ADCC, their complementary mechanisms of action resulted in the significantly enhanced antitumor activity [142]. Ramalingam et al. carried out a phase II randomized, double-blinded, and placebo-controlled study to assess the efficacy of vorinostat, in combination with carboplatin and paclitaxel, as a first-line therapy for advanced NSCLC. Vorinostat, a histone deacetylase inhibitor, exerted anticancer effects by both histone and nonhistone-mediated mechanisms. A confirmed response rate (CRR) of 34% was recorded in 94 patients and the overall survival increased from 9.7 months to 13.0 months [143]. A few combination therapies involving mAbs are currently in various phases of clinical trials and have been stated in Table 8.7.

8 Clinical Studies

Over the past decades, lung cancer has been regarded as one of the leading cause of cancer-related mortality in both men and women. Several mutations, like the occurrence of inversions in the short arm of the chromosome that juxtaposes echinoderm microtubule-associated protein-like 4 (EML4) with ALK and produces EML4-ALK–fusion tyrosine kinases, substitution of threonine at 790 to methionine (T790M), escaping the elimination by immune system through programmed death (PD-1) pathway, etc., have been commonly encountered in various phases of clinical studies [155–159].

Crizotinib, a multitargeted TKI was approved by the USFDA in August 2011 for the treatment of advanced NSCLC. The drug exhibited activity against c-MET, ALK, and ROS1 in advanced NSCLC cases that were positive for the ALK rearrangements. About 65–74% of the patients benefitted from this therapy and demonstrated a median progression-free survival rate of 7.7–10.9 months [155, 160]. Other small-molecule TKIs, such as crizotinib, imatinib, erlotinib, and gefitinib, were also approved for the treatment of lung cancer. But, these drugs exhibited low cerebrospinal fluid (CSF)-to-plasma ratios since the central nervous system (CNS) remains one of the dominant sites of progressive tumor burden during chemotherapy with crizotinib and other molecules [155, 160]. The first-generation TKI's, gefitinib, and erlotinib are reversible small-molecule ATP analog, originally designed to inhibit the tyrosine kinase activity of the wild-type EGFR. These were found to be most effective in advanced NSCLC, with a median overall survival period of approximately 19–36 months. But, these first-generation TKIs were associated with side

effects like skin rash and diarrhea due to the inhibition of wild-type EGFR present in the skin and gastrointestinal organs. Furthermore, amplification in HER2 and c-MET, mutation in PIK3CA and BRAF, and loss of NF1, T790M were observed as the most common mechanisms of resistance by the tumor cells in more than 50% of the patients exhibiting disease progression. The T790M mutation is believed to provide resistance against the reversible first-generation TKIs through steric hindrance and increased affinity toward ATP. The second-generation, irreversible EGFR TKIs, such as afatinib and dacomitinib, have proven effective against untreated, EGFR mutant lung cancer. But, as a monotherapy, they have failed to overcome the T790M-mediated resistance in patients, because the concentrations at which these irreversible TKIs overcome the T790M activity in preclinical trials cannot be achieved in humans due to the dose-limiting toxicity related to the nonselective inhibition of the wild-type EGFR. AstraZeneca (Macclesfield, UK) developed an oral, third-generation, irreversible, small-molecule inhibitor (AZD9291) to target the T790M-resistant mutant forms (EGFRm+) with selectivity over the wild-type EGFR. AZD9291 has a chemical structure distinct from the other third-generation TKIs, WZ4002 and CO-1686. This drug acts by binding to the EGFR kinase and targeting the cysteine-797 residue in the ATP-binding site through the formation of an irreversible covalent bond. In the phase I of clinical trials, the drug was found to be 200 times more potent against the T790M mutant than the wild-type EGFR [156].

Several next-generation ALK inhibitors that are more potent than crizotinib, have entered various clinical studies and can overcome the most common mutations conferring resistance to ALK such as Leu1196Met. Among the eight next-generation ALK inhibitors that have entered the clinical trials, three molecules, namely, ceritinib, alectinib, and brigatinib have demonstrated a robust activity in patients with ALK-positive NSCLC. Alectinib has also shown its antitumor activity in patients resistant to crizotinib. 125 subjects were screened during a phase II study in patients with NSCLC, wherein 87 ALK-positive candidates whose disease progressed after crizotinib, were enrolled. The results of this study showed that alectinib was effective in patients suffering from ALK-positive NSCLC and was well tolerated, resulting predominantly in grade 1 or 2 adverse events with improved quality of life. Alectinib also exhibited several potential advantages in terms of both efficacy and tolerability. The median duration of response was prolonged with alectinib (13.5 months) as compared to ceritinib (8.2 months) and brigatinib (9.3 months), respectively. Thus, patients who did not respond to the treatment with crizotinib could be treated with the aforementioned ALK inhibitors, alectinib also resulted in intracranial disease control in 85% and 56% of the patients, at 12 and 24 weeks, respectively [160].

Apart from the mutations occurring in lung cancer, tumors can also escape elimination by the immune system through the activation of inhibitory feedback loops (also known as immunological brakes), which are essential to avoid autoimmune events, and can thus bypass tumor rejection and T-cell activation. The PD-1 and B7.1, also known as CD80 receptors, follow this inhibitory pathway and their activation has been observed in several cancer types including the lung cancer [157–159]. Nivolumab is a fully human, IgG4 immune checkpoint inhibitor antibody, which

Table 8.7 Combination therapy with monoclonal antibodies against EGFR

Sr. no.	mAb	Type/class	EGFR-binding domain	Development phase	In combination with	Therapy	References
1	Cetuximab	Chimeric human murine IgG1	Domain III	III	Only cetuximab	For maintenance after platinum-based chemotherapy during first-line treatment of NSCLC	[144]
					With cisplatin and vinorelbine	First-line treatment of advanced NSCLC	[145]
					With docetaxel and pemetrexed	Treatment of recurrent or progressive NSCLC	[146]
					With carboplatin, docetaxel, and paclitaxel	First-line treatment of patients with advanced/ metastatic NSCLC	[147]
2	Nimotuzumab	Humanized IgG1 obtained from murine IgG2a mAb	Domain III	II	Nimotuzumab plus gefitinib	Randomized Phase II Study of gefitinib and nimotuzumab versus gefitinib in patients with advanced non-small cell lung cancer: dual-agent molecular targeting of EGFR (DATE)	[148]
3	Panitumumab (ABX-EGF)	Recombinant fully human IgG2 mAb	Binds EGFR and inhibits EGF induced RTK phosphorylation	II	In combination with paclitaxel and carboplatin	Treatment of advanced non-small cell lung cancer (NSCLC)	[149]
					Carboplatin, pemetrexed, and panitumumab	Patients with advanced nonsquamous k-ras wild-type NSCLC	[150]
4	Pertuzumab	Recombinant humanized mAb	Domain II of HER2	II	Erlotinib and pertuzumab	Patients with relapsed non-small cell lung cancer	[151]
					Only pertuzumab	Patients with advanced non-small cell lung cancer, which has progressed after prior chemotherapy	[152]
5	Necitumumab	Recombinant human IgG1 monoclonal antibody used as an antineoplastic	Domain III	II	Gemcitabine-cisplatin chemotherapy plus necitumumab	First-line treatment of participants with squamous lung cancer	[153]
6	Trastuzumab Emstatine	–	–	II	Only trastuzumab and emstatine	Participants with human epidermal growth factor receptor (HER)2 immunohistochemistry (IHC)-positive, locally advanced or metastatic non-small cell lung cancer (NSCLC)	[154]

binds to the PD-1 receptors on activated immune cells and thereby inhibits its interaction with PD-L1 and PD-L2 ligands. This event attenuates inhibitory signals and promotes antitumor responses by the host. A phase II clinical study employing 140 patients was conducted, in which, 117 (84%) patients were treated with nivolumab (Bristol-Myers Squibb, Princeton, NJ, USA), as an injectable solution (100 mg; 10 mg/mL) for a period of 100 days. Patients received nivolumab as an intravenous infusion at the concentration 3 mg/kg, every 2 weeks (1 cycle) until the disease progression or unacceptable toxic effects appeared. Nivolumab showed activity in patients with advanced, refractory, and squamous NSCLC and was associated with a manageable safety profile [157].

An early phase clinical trial was initiated with an engineered, humanized IgG1 monoclonal anti-PD-L1 antibody, atezolizumab (MPDL3280A; F Hoffmann-La Roche/Genentech). This antibody acts by blocking PD-L1–PD-1 and PD-L1–B7.1 interactions, which results in the overhauling of T-cell activity and enhancing T-cell priming. POPLAR, a multicenter, randomized, open-label, all comer phase II trial, was carried out at 61 academic medical centers and community oncology practices, across 13 countries in Europe and North America. It was primarily designed to investigate the efficacy and safety of atezolizumab versus docetaxel as the second-line and third-line treatments in NSCLC, and to further assess the predictive value of PD-L1 expression level in tumor cells and tumor-infiltrating immune cells. Accordingly, patients received intravenous atezolizumab (1200 mg fixed dose) or docetaxel (75 mg/m^2) every 3 weeks, on day 1 of each 3-week cycle. Docetaxel was given until disease progression or unacceptable toxicity was observed. No docetaxel-to-atezolizumab crossover was allowed. Results indicated that patients with either squamous or nonsquamous NSCLC showed significant improvement in their overall survival upon treatment with atezolizumab as compared with patients who received docetaxel. Also, atezolizumab was well tolerated and exhibited a safety profile that was consistent with the previous studies [158].

Further, a combination of immunotherapy and chemotherapy was evaluated for its potential to synergistically improve the anticancer activity of the individual drugs. Currently, the standard first-line therapy for patients with advanced nonsquamous NSCLC is platinum-doublet chemotherapy. With the exception of bevacizumab, the addition of a third agent to the platinum-doublet chemotherapy has not improved the progression-free or overall survival rate as compared to the platinum-doublet chemotherapy alone in randomized studies. A study was carried out with pembrolizumab, a humanized, monoclonal antibody against PD-1 that prevents PD-1 from binding to its ligands, PD-L1 and PD-L2. A randomized KEYNOTE-021, phase II study was carried out at 26 academic medical centers in the USA and Taiwan, in patients with chemotherapy-naive, advanced nonsquamous NSCLC. A combination of pembrolizumab and pemetrexed was administered to the patients, wherein the subjects received four cycles of pembrolizumab (200 mg), over 30 min. Further, the chemical drug, pemetrexed was administered at a concentration of 500 mg/m^2 over 10 min, and carboplatin at a dose of 5 mg/mL per min was administered over 15–60 min, intravenously every 3 weeks in the order listed, followed by pembrolizumab for 24 months and optional indefinite pemetrexed maintenance therapy. 123 (56%) patients from the

USA and Taiwan met the eligibility criteria and were randomly distributed for different treatment regimes. 60 patients (49%) were treated with pembrolizumab along with carboplatin and pemetrexed, while 63 patients (51%) were treated with carboplatin and pemetrexed alone. Addition of pembrolizumab to carboplatin and pemetrexed followed by pembrolizumab for 2 years and indefinite pemetrexed maintenance therapy significantly improved the proportion of patients who achieved an objective response as compared to those receiving carboplatin and pemetrexed alone. The median progression-free survival time in the pembrolizumab plus chemotherapy group was 13 months, while the progression-free survival recorded in the chemotherapy group was 8.9 months [159].

9 Conclusion

Lung cancer has been a long-term challenge and still demands newer treatment modalities for its eradication. Availability of safe and effective treatment options has been hampered due to drug resistance and concurrent mutations at various levels. However, research over decades has offered various therapies that have yielded promising results in preclinical and clinical trials. Today, our understanding about cancers has reached greater depths and has enabled the prognosis of various cancer types. A greater understanding of the molecular biomarkers of lung cancer as well as an in-depth understanding of specific receptors overexpressed in this form of cancer will enable the provision of personalized therapies for eradicating this dreadful disease.

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